Rheology and microscopy of low calorie mayonnaise-type emulsions made with stearic acid- modified maize and teff starches

by

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Submitted in partial fulfillment of the requirements for the degree

Master of Science: (MSc-Agric) Food Science and Technology

in the

Department of Food Science

Faculty Natural and Agricultural Sciences

University of Pretoria

Pretoria

Republic of South Africa

April 2011

Declaration

I declare that this dissertation submitted at the University of Pretoria for the degree: MSc-Agric: Food Science and Technology has not been submitted by me for a degree at any other University or Institution of higher education

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ACKNOWLEDGEMENTS

After passing all the ups and downs of this project, at this time I would like to acknowledge all the individuals and organizations who played a vital role in the completion of this project and who supported me all the way through.

My earnest appreciation goes to two individuals for whom I have a great respect:

My supervisor, Dr. Naushad Emmambux, for his expert guidance, keen interest in this project and patient supervision. His challenging ideas, honest comments and knowledgeable enthusiasm into this field of study enabled me to complete the project to the best of my ability.

Dr. Gyebi Duodu, my co-supervisor, for his useful comments, support and words of encouragement.

I would also like to thank:

The Ethiopian Institute of Agricultural Research and the Rural Capacity Building Project, Ministry of Agriculture (Federal Democratic Republic of Ethiopia) for the financial support. Mr. Alan Hall (Department of Microscopy and Microanalysis, University of Pretoria) for his support with the microscopy conducted for this work.

The academic and technical staff and students of the Department of Food Science, University of Pretoria for their helpful information and words of encouragement.

My soon-to-be wife, Bethlehem Mezgebe, for her unfailing support and loving patience. I love you dearly. My mother, brothers and sisters for loving me unconditionally and giving me all the help they can.

All members of the Ethiopian Orthodox Tewahido Church in Pretoria, South Africa for their support and encouragement.

Finally, my Creator, the Heavenly Father, for giving me the health, time, opportunity, His mercy and never ending love. The credit is all His.

ABSTRACT

Excessive consumption of diet rich in fat has been identified as a risk factor for lifestyle diseases. Fat replacers which can imitate the functional properties of fats with lower or no calorie contribution have been developed to produce low-calorie foods. Starch (mostly modified) can be used as fat replacer in low fat foods. Teff (a cereal indigenous to Africa, which is underutilized and underresearched) and maize starches modified with stearic acid (a naturally occurring fatty acid), has resulted in a paste with reduced gelling ability and higher viscosity in previous research. This suggests that these modified starches may have potential to be used as fat replacers. This project investigates the effect of teff and maize starch pastes modified with stearic acid as fat replacer on the rheological properties (flow properties and viscoelastic behaviour), microstructure (using light and confocal laser scanning microscopy), and freeze-thaw and high temperature stability of low-calorie mayonnaise type emulsions (LCMTE).

Starch suspensions (10% w/v of starch in water) containing stearic acid (1.5%) were pasted for an extended holding time of 2 hr at 91 °C in a Rapid Visco Analyser (RVA) and used to prepare LCMTE with the oil replaced at 50%, 80% and 100% in comparison to a full fat mayonnaise. All samples exhibited a shear thinning behaviour and yield stress in terms of flow properties. Modification of teff and maize starches with stearic acid decreased the yield stress and viscosity of the LCMTE compared to unmodified starches. LCMTE with maize starch had higher yield stress and viscosity compared to LCMTE with teff starch. The yield stress value decreased on increasing the level of oil replacement from 50% to 80% and then increased when the oil replacement was 100% except for LCMTE with modified teff starch. The yield stress value for LCMTE with modified teff starch decreased with increasing level of oil replacement. Storage for 8 days was found to increase the yield stress and viscosity except for LCMTE with modified teff starch.

Viscoelastic properties of mayonnaise were characterized using dynamic oscillatory shear test and it was observed that all samples behaved like solid with storage modulus (G') greater than loss modulus (G") and tanδ < 1. Generally LCMTE with modified starches had lower G', G" and tanδ than LCMTE with unmodified starches, suggesting less gelling behaviour of teff and maize starch pastes modified with stearic acid.

The optical and laser scanning microscopy images showed all samples to consist of oil droplets dispersed in aqueous phase except the LCMTE at 100% oil replacement level. LCMTE had oil droplets of smaller size (1-10 μm) compared to the full fat mayonnaise (5-10 μm). LCMTE with modified starches had larger size oil droplets compared to LCMTE with unmodified starches. LCMTE with maize starches seemed to have smaller oil droplets compared to LCMTE with teff starches especially with the modified starches with stearic acid. The smaller size oil droplets of LCMTE with unmodified starches can be related to the higher yield stress and viscosity values compared to the LCMTE with modified starches which have larger size oil droplets.

All the LCMTE with modified and unmodified starches were found to be more stable to freeze-thaw cycles and high temperature storage compared to full fat mayonnaise. Generally, LCMTE could be produced with modified and unmodified teff and maize starches, the properties being dependent on the level of oil reduction. At 50% oil replacement modified and unmodified teff and maize starch with stearic acid could produce LCMTE. When the oil content was further decreased to 80% and 100% only the LCMTE with modified starches were similar to the full fat. The LCMTE with unmodified starches were found to have non-pourable gel-like behaviour with higher yield stress and viscosity values than the full fat mayonnaise. Teff and maize starches modified with stearic acid (1.5%) could be used as fat replacers in mayonnaise replacing up to 100% oil and this can substantially decrease the calorific value of mayonnaises. The reduction in the calorie may contribute to weight loss, and prevention of cardiovascular diseases. The starch pastes modified with stearic acid may also have a potential to be used as emulsifiers.

Keywords: Fat replacer; Low-calorie mayonnaise type emulsions; Amylose-stearic acid complexe; Teff starch

Abbrevations: LCMTE

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1. INTRODUCTION

Fat has important nutritional and functional roles in food. However, excessive consumption of diet rich in fat has been identified as a risk factor for lifestyle diseases, for example obesity, cardiovascular disease (Van Gaal et al., 2006), cancer (Dogan et al., 2007), type 2 diabetes (Van Dam et al., 2002), and coronary heart disease (Hu et al., 1997). Thus, production of low-calorie foods have been considered as a solution for the health problems related with high fat food consumption. However, the production of low-calorie foods is not straightforward; because reduction of fat from foods would result in unacceptable sensory qualities, for example dilute flavour and poor texture in cheese (Drake et al., 1996). This is because fat contributes key sensory and functional characteristics such as mouthfeel, taste, creaminess, palatability, lubricity, juiciness, heat transfer and increased feeling of satiety (Pearson & Gillett, 1999). Thus, fat replacers appear to be potential solutions to offer substantial reduction of fat without compromising eating quality (Nabors, 1992).

Fat replacers can imitate the functional properties of fats with lower or no calorie contribution (Grossklaus, 1996). Starch, one of the most available biopolymer can be used as fat replacer in low fat foods (Giese, 1996). Due to its capacity to stabilize a substantial amount of water, starch can mimic the creamy mouthfeel of fats. Although native starch can sometimes be used as fat replacer, starches are commonly modified through physical and/or chemical techniques to be used as fat replacers (Jane, 1992; Akoh, 1998). The use of chemicals to modify starch, in spite of its effectiveness in changing the starch functionality has raised health concerns especially for food application. Thus, there is a demand to use naturally occurring compounds to modify starch. Modification of teff (a cereal indigenous to Ethiopia and considered as one of the lost crops of Africa, which is underutilized and under-researched) and maize starches with stearic acid, a naturally occurring fatty acid, has resulted in a paste with reduced gelling ability and higher viscosity (D'Silva, 2009). These properties suggest that teff and maize starch pastes modified with stearic acid have potential to be used as fat replacers.

Mayonnaise is considered as a high fat food with up to 80% oil traditionally. It is widely used as sauce in burgers and fast food outlets. Thus because of its high fat content, mayonnaise is one potential food product for fat reduction to produce low-calorie product.

2. LITERATURE REVIEW

In this chapter, the current literature on the manufacture, rheology and stability of mayonnaise is first discussed. Then the types and use of fat replacers in different high fat foods with special emphasis on starch fat replacers are reviewed.

2.1 Mayonnaise

2.1.1 Definition and Use

Mayonnaise is a semi-solid, oil-in-water food emulsion. It consists of edible vegetable oil, vinegar, egg yolk, sugar, salt, and other optional ingredients, for example spices. According to the South African definition, mayonnaise shall contain at least 52% edible vegetable oil by volume (Ministry of Health, Act No. 54, 1972). The United States law requires that mayonnaise contain at least 65% vegetable oil by weight (US code of Federal Regulation, 2006). The only permitted emulsifying agent in mayonnaise in the United States is egg yolk (Krog et al., 1985).

Mayonnaise is one of the most widely used sauces or condiments in the world today. It is used on French fries, hot dogs, locos, boiled potatoes, sandwiches, tuna salad, as salad dressing, served with dishes such as takoyaki, tyroleanham cordon bleu, and as base for many other chilled sauces and salad dressings, for example, tartar sauce, thousand island dressing, fry sauce, mayonesa, and ranch dressing.

2.1.2 Manufacture of Mayonnaise

Mayonnaise is prepared by mixing all the ingredients other than the oil (the aqueous phase) first. Then the oil is added little by little while continuously stirring the whole mixture at high speed. The high speed stirring is used to form small oil droplets in a continuous water phase with sufficiently high viscosity to prevent coalescence of the oil droplet. Oil and vinegar (a dilute concentration of acetic acid in water) (the two main components) in mayonnaise are immiscible liquids. Oil and water molecules are energetically unfavourable to react (McClements, 2000). The aqueous phase (vinegar) consists of polar molecules, namely water and acetic acid. These two molecules have strong hydrogen bonding between them because the oxygen atom in water and the H^+ ions in acetic acid attract each other. On the other hand oil molecules have also strong hydrophobic attractions to themselves. The intermolecular interaction in a water-oil-acetic acid mixture will favour more an interaction of water-acetic acid > oil-oil > oil-water interaction. Thus whisking a mixture of oil-acetic acid and water will always produce two distinct separated phases, a layer of oil on top of water-acetic acid mixture. An emulsifier is therefore required to reduce the surface tension between oil and water. In mayonnaise egg yolk is used as an emulsifier to produce kinetically stable emulsion (Kontogiorgos et al., 2004).

Egg yolk has an emulsifier known as lecithin, chemically named as diacyl-phosphatidyl choline (Figure 2.1). This can function as a natural emulsifier (Dickinson, 1993). An emulsifier is a surface active molecule which functions by lowering the interfacial energy between the two immiscible liquids (oil and water). It also adsorbs to the surface of emulsion droplets to form a protective coating that prevents the oil droplets from coalescing. Emulsifiers are amphiphilic, having both hydrophobic and hydrophilic ends. Thus they have properties which allow them to interact with both the lipid and aqueous phases of the mixture. Non-polar ends of the emulsifier align themselves within the lipid phase (the oil); while the polar ends align in the water phase (Figure 2.2).

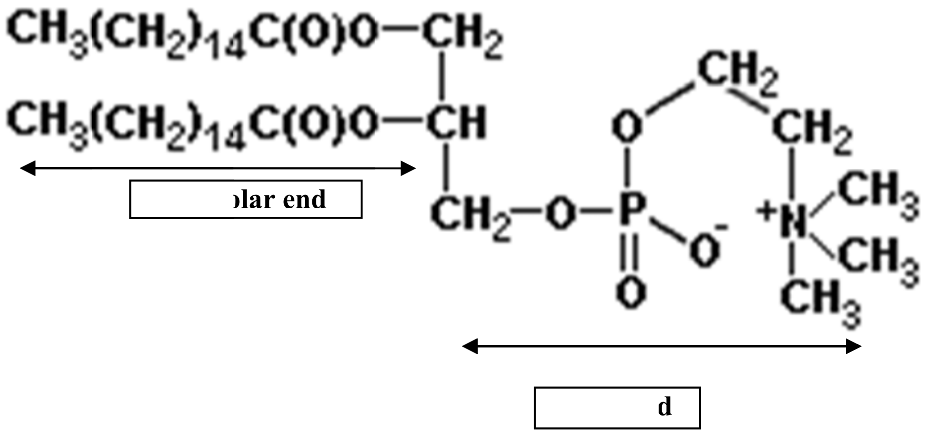


Figure 2.1: Molecular structure of Lecithin (diacyl-phosphatidyl choline) showing the polar (on the right) and non-polar (left) ends (Duke & Chapoy, 1976).

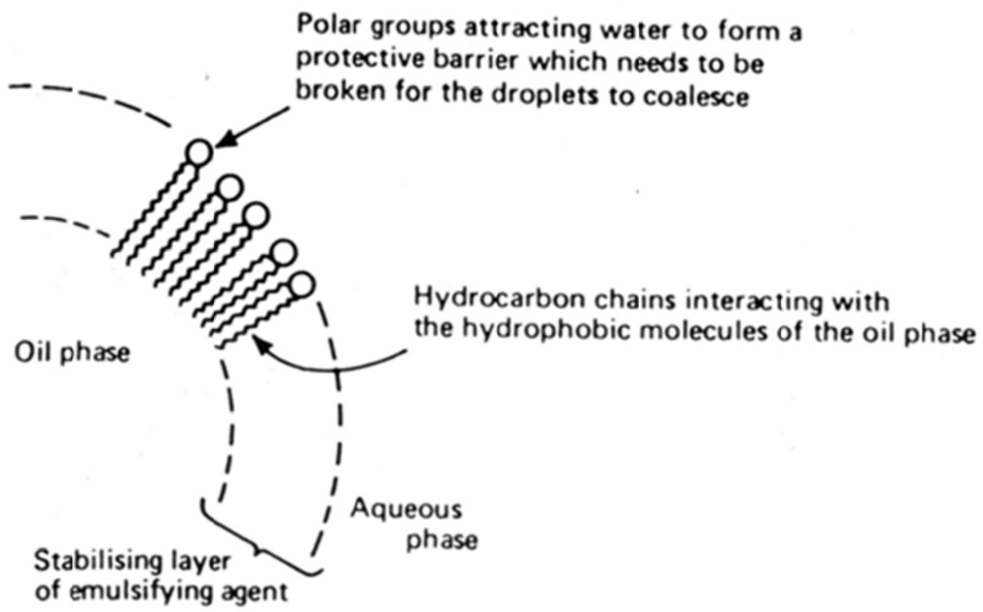


Figure 2.2: Alignment of emulsifier molecules around oil droplet to stabilize an oil-in-water emulsion.

Each oil globule in mayonnaise suspension is coated by at least one layer of lecithin, which allows it to mix freely with the aqueous phase because the hydrophilic part contacts the water while the hydrophobic part faces the oil. The droplets do not readily coalesce because the hydrophilic ends of the emulsifier protruding from their surface usually acquire small electric charges and consequently repel one another. The strength of the interactions between oil droplets depends on the van der Waals attractions, which are balanced by electrostatic and steric repulsion. The quality of the emulsion will depend on the right balance between the attraction and repulsion forces.

As a component of mayonnaise, oil contributes to the high viscosity (texture), appearance, flavour and the thick creamy mouthfeel. As mayonnaise is produced with a shear force applied using a colloidal mill or homogenizer the oil phase will disperse into tiny droplets that remain throughout the water phase. Thus, the water phase is used to suspend the dispersed oil globules. In full fat mayonnaise which contains about 80% oil, the size of the oil droplets is usually between 1 and 5µm in diameter (Andrew, 1999).

In an ideal emulsion consisting of spherical droplets packed together within the continuous phase, the dispersed phase can only account for a maximum of 74% of the total volume. In mayonnaise, however, the oil may account for 75% or more of the total volume. The close packing of the oil droplets also allows them to interact very strongly with one another. The high oil volume in combination with the strong interaction of the oil droplets gives traditional mayonnaise its high viscosity (Harrison & Cunningham, 1985). Mayonnaise with 80% oil approaches the limit for a stable oil-in-water emulsion.

Vinegar in mayonnaise is also used for flavouring and as an acidifying agent. The lower the pH of the product the longer the shelf life is. Salt and sugar can also be added to mayonnaise as optional ingredients and their purpose is for flavouring and preservation by decreasing the water activity.

2.1.3 Rheology of Mayonnaise

Rheology is the science of material deformation and flow in response to the application of a force. Mayonnaise is a viscous product with a thick creamy mouth-feel. Thus the rheology of mayonnaise (flow properties) gives an indication of its quality. The flow properties of mayonnaise also influences the consumers' attitude as it affects sensory properties like texture and flavour and functional properties such as application on salads, French fries or other meals (Štern et al., 2001). As reviewed by Juszczak et al. (2003) the rheological properties of mayonnaise are mostly determined by the fat content, the emulsifier and the presence of stabilizers and thickeners.

The Power Law equation: $\sigma = K(\dot{\gamma})^n$ or the Herschel-Bulkely equation $\sigma = K(\dot{\gamma})^n + \sigma_{0;}$ [where σ is shear stress (Pa), $\dot{\gamma}$ is shear rate (1/s), K is the consistency index (Pa s^n), n is the flow behaviour index, and σ_0 is yield stress (Pa)] have been widely used to describe flow properties of mayonnaise and salad dressings (Peressini et al., 1998; Guilmineau & Kulozik, 2007; Batista et al., 2006). The consistency index (K-value) is an indication of the viscosity of the material. The flow behaviour index (n-value) indicates the flow property of the material. For Newtonian fluids $n=1$ and the consistency index $(k) = \mu$ (viscosity), $n < 1$ for shear thinning materials and, $n > 1$ for shear thickening materials.

Mayonnaise is a shear thinning non-Newtonian fluid, where the viscosity decreases on increasing the shear rate (Figure 2.3). During the shearing process the droplets will be oriented in the shear direction which lowers its flow resistance and with increase in shear rate, the viscosity will decrease progressively.

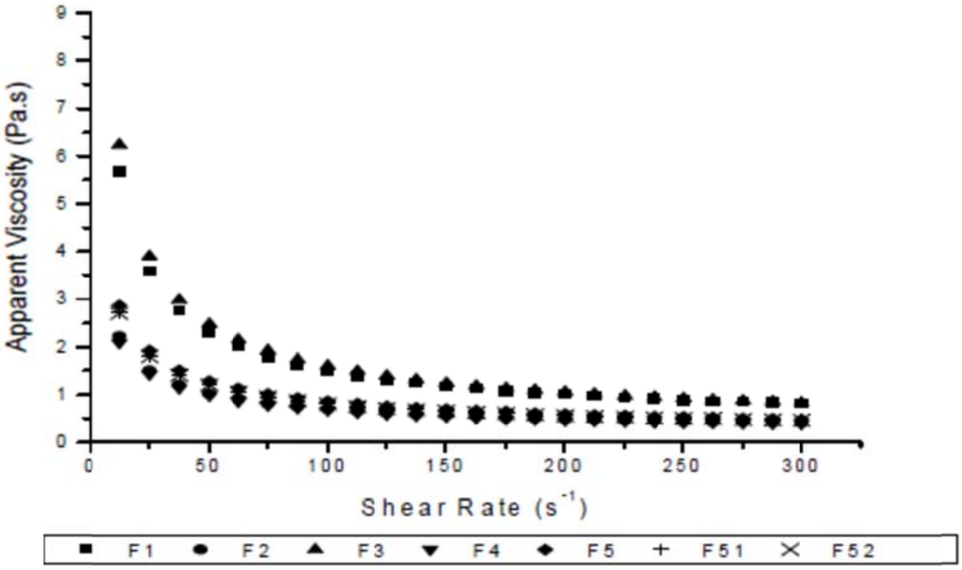


Figure 2.3: Apparent shear viscosity curves of five mayonnaise formulations with the proportion, respectively: water/soy oil/green banana pulp: F1 (0.10/0.20/0.70), F2 (0.20/0.20/0.60), F3 (0.10/0.25/0.65), F4 (0.20/0.25/0.55) and F5 (0.15/0.225/0.625) mayonnaise formulations (Izidoro et al., 2009).

Mayonnaise has a yield stress. Yield stress can be defined as the minimum shear stress value required to initiate flow. A material with yield stress like mayonnaise begins to flow only when the external force applied is greater than the yield stress value, which may range from 25.8 Pa (Mun et al., 2009) to 43.64 Pa (Su et al., 2010). The yield stress value of mayonnaise is of great importance to the product acceptance among consumers (Juszczak et al., 2003). When used as salad dressing mayonnaise must have a proper ability to keep non-fluid on the salad surface and this happens only if it has a yield stress. Yield stress also gives stability to food emulsions like mayonnaise in low-stress situations (lower than the yield stress) like those of storage and transportation (Izidoro et al., 2008).

Mayonnaise shows a thixotropic behaviour (Kozin & Darchiev, 1972). Thixotropy may be defined as change in structural strength due to mechanical load. Thixotropic behaviour means a reduction of the structural strength during a shear load phase followed by rapid and complete structural regeneration during rest period. The area between the upward flow curve and the downward flow curve may be considered as a measure of thixotropy (Figure 2.4). A lower thixotropic area corresponds with the ability of a material to quickly rebuild the damaged structure after the removal of shear forces (Abu-Jdayil, 2003).

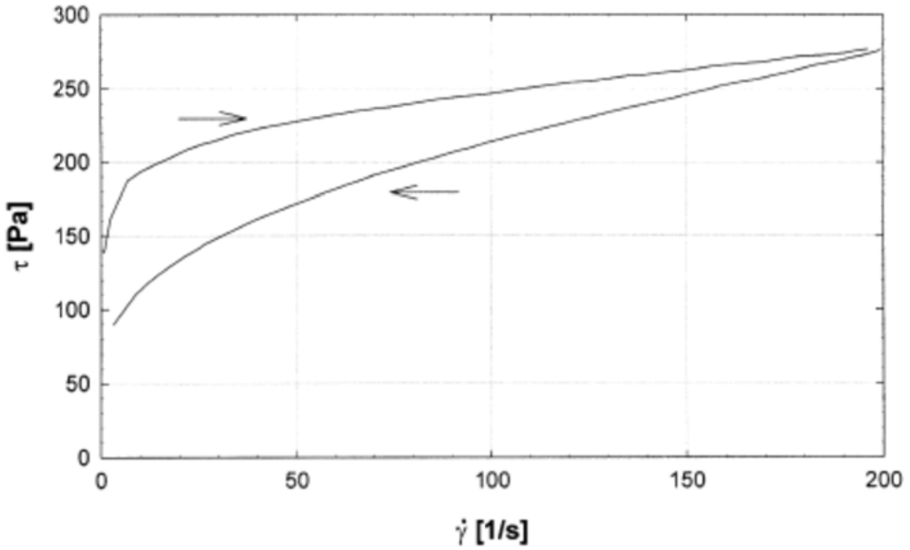


Figure 2.4: Typical flow curve for mayonnaise: up-curve →(increasing the shear rate); ← down-curve (decreasing the shear rate) (Štern et al., 2001).

In emulsions a large decrease in viscosity is observed above a critical shear-rate/shear-stress (Barnes, 1994; Otsubo & Prudhomme, 1994). This drop in viscosity (shear-thinning) is seen at a lower shear rate/stress when apparent wall slip occurs; showing in some cases a false pseudo-Newtonian plateau at intermediate shear stresses (Franco et al., 1998). Apparent wall slip occurs in multi-phase systems when the dispersed phase migrates away from solid boundaries, like the walls of the sensor system in a rheometer, leaving a thin layer of lower-viscosity liquid. The thin layer liquid will act as a lubricant between the material and the sensor system of the rheometer. This leads to an apparent decrease in the measured bulk viscosity (Goshawk et al., 1998). Wall slip phenomenon arises from steric, hydrodynamic, viscoelastic, chemical and gravitational forces acting on the disperse phase immediately adjacent to solid boundaries (sensor system of the rheometer) (Barnes, 1995). Ma and Barbosa-Cànovas (1995) characterized two batches of mayonnaise in parallel-plate geometry and they observed apparent wall slip for all samples in shear flow which was attributed to the formation of a boundary layer a few tens of nanometres thick. In oscillatory shear, however, there was no evidence of apparent wall slip. Bower et al. (1999) used an optical microscope to observe the shearing process in mayonnaise. They observed all the droplets to move together with the same velocity as the moving bottom disc in shear rates below 1 s^{-1}. Thus the emulsion was slipping at the interface between the fluid and the top stationary glass disc. As the applied shear was increased above 1 s^{-1}, relative movement became apparent between adjacent droplets and the material appeared to move as a continuum with little or no relative slip at the top and bottom glass interface. Oscillatory tests were performed by Mancini et al. (2002) on mayonnaise at different gaps (1.00 and 1.5 mm) using different sensor geometries (plate-plate and cone-plate) and found that slip effects were not significant in the linear viscoelastic range and in the non-linear region close to the critical strain. Ma and Barbosa-Cànovas (1995) and Plucinski et al. (1998) have also reported the absence of slippage effects during dynamic mechanical experiments performed on mayonnaise under low strain conditions (linear region).

Slip is often more pronounced when an emulsion is formed by large droplets, or when it is weakly flocculated and the flocs behave as individual large droplets under the action of gravity. For this reason, plate-plate or cone-plate geometries (that is horizontally aligned sensor systems) are more vulnerable to slip (Barnes, 1995). Obviously, in order to investigate the true internal microstructure of materials, slip effects must be avoided or corrected. Generally it is assumed that a non-slip flow curve may be obtained either in vane geometry (Carnali & Barnes, 1990), using a sensor system with rough surfaces (White et al., 1991; Buscall et al., 1993; Gregory & Mayers, 1993; Mas & Magnin, 1994) or even sometimes changing the nature of the sensor system material (using glass instead of steel) (Otsubo & Prudhomme, 1994).

Small amplitude shear tests, conducted within the linear viscoelastic (LVE) region, causing minimum disturbance to the structure are useful for investigating the structural properties of materials. The results from small amplitude oscillatory shear tests are expressed in terms of the elastic modulus (G') and loss modulus (G"). If G' > G", the material will exhibit a solid like behaviour (i.e. deformation in the linear range will be essentially elastic or recoverable); however, if G" > G', the material will behave like a liquid (i.e. the energy used to deform the material will be viscously dissipated). Mayonnaise has been shown to be viscoelastic, showing viscous and elastic behaviour simultaneously (Gallegos et al., 1992; Franco et al., 1997; Peression et al., 1998). The viscoelastic behaviour of mayonnaise is attributable to a network formed between lipoproteins which are adsorbed around neighbouring oil droplets (Muńoz & Sherman, 1990). Peressini et al. (1998) tested four commercial traditional and dietetic (has low caloric content naturally or through process) mayonnaise samples at frequencies ranging between 0.1 and 10 Hz at 1% strain and all samples exhibited viscoelastic behaviour with storage modulus (G') greater than the loss modulus (G"). Oscillatory tests were carried out on full fat and low-fat mayonnaises in a frequency range of 0.1 – 60 Hz (Liu et al., 2007) and all samples showed viscoelastic behaviour with a storage modulus (G') greater than the loss modulus (G"). Higher oil content produces a significant increase in the elastic behaviour of mayonnaise (higher values of G') (Gallegos et al., 1992; Ma & Barbosa-Cànovas, 1995). With increase in temperature the elastic behaviour (storage modulus) of mayonnaise decreases (Guerrero & Ball, 1994).

2.1.4 Stability of Mayonnaise

a) Physical Stability

Emulsion stability usually involves preventing the physicochemical mechanisms responsible for emulsion instability which are droplet coalescence, flocculation and creaming (Mun et al., 2009; McClemets & Demetriades, 1998). Creaming denotes the upward movement of oil droplets under the action of gravity; flocculation is the clustering of oil droplets; and coalescence means the spontaneous joining of small droplets into larger ones. In mayonnaise, coalescence is the main problem (Dickinson & Stainsby, 1982, 1987). In highly concentrated emulsions like full fat mayonnaise the oil droplet will coalesce forming larger size oil droplets and finally phase separation will occur (Abu-Jdayil, 2003).

Stabilizers are added to emulsions to confer long term stability. Stabilizers are normally biopolymers, polysaccharides or protein. The main stabilizing action of polysaccharides is through modification of viscosity of aqueous phase, specifically by increasing viscosity of the continuous phase and the yield stress and retarding the movement of the droplets (Dickinson, 2003; Paraskevopoulou et al., 2005). Proteins contain a mixture of hydrophilic and hydrophobic groups inducing intermolecular bonds more readily, conferring both steric stabilization and surface activity (Martinez et al., 2007). Thus, proteins can act as stabilizer and emulsifier.

b) Microbial Stability (Safety)

Reports of food borne illness associated directly with the consumption of commercially prepared acidic sauces and dressings are rare (Smittle, 2002). Preservation of acidic sauces and mayonnaise-based salads predominantly focuses on acidification by addition of organic acids and reducing water activity by addition of soluble compounds (Vermeulen, 2008). In mayonnaise, vinegar and salt are the principal preservatives against pathogenic bacteria.

Mayonnaise has a low pH (3.0 – 4.2) with the highest tolerated value of 4.5 (ICMSF, 2005) due to the addition of acetic acid (vinegar). Generally, microbial vegetative cells are rapidly killed by acetic acid while the endospores of spore forming bacteria remain viable. In case germination of these spores occurs; growth of the cells will be inhibited at such low pH (Smittle, 1977).

The percentage of salt added to mayonnaise is not fixed by regulations, and ranges from 1% to 12% on the aqueous phase, leading to a water activity of 0.95 to 0.93 (Karas et al., 2002; ICMSF, 2005). The addition of the soluble compounds, salt and sugar, which decrease the amount of available water for the microorganisms will have a major influence on their survival (Vermeulen, 2008).

Because commercial mayonnaise is formulated carefully with highly acidic ingredients and pasteurized eggs (free of Salmonella and other pathogenic bacteria), it is generally regarded as safe product from a microbiological perspective.

c) **Chemical stability**

There is limited literature on the chemical stability of mayonnaise and emulsions in general. The limited studies available deal with spoilage due to lipid auto-oxidation although chemical stability refers to the alterations in the chemical structure of the ingredients such as oxidation, lipolysis, proteolysis, or polymerization (McClemets & Demetriades, 1998). Like all high fat foods mayonnaise is also susceptible to auto-oxidation of unsaturated and polyunsaturated fats in the oil (Depree & Savage, 2001). The high iron content of egg yolk (40 μg/g) could contribute to this, as it is well known that transition metals accelerate oxidation reaction by hydrogen abstraction and peroxide decomposition, which results in the formation of free radicals (Decker, 1998). Mayonnaise also has large area of the oil exposed to the aqueous phase which may contain dissolved oxygen and the blending process tends to introduce air bubbles which increase the amount of oxygen which is a necessary reactant for the oxidation. In mayonnaise and similar emulsions, oxidation appears to initiate at the droplet interface, which means that small droplet size may promote the initiation of the oxidation, but once oxidation has been initiated propagation is independent of droplet size (Jacobson et al., 2000b).

The stability of mayonnaise to oxidation was found to be dependent on the oil type used. Hsieh and Regenstein (1992) used fish oil, corn oil and soy oil to prepare mayonnaise samples of 70% oil by weight and during 8 weeks storage at 30 °C, they found that mayonnaise made with fish oil oxidized very rapidly, mayonnaise with corn oil was less susceptible to oxidation and mayonnaise with soy oil was the least susceptible to oxidation. It is possible that the soy oil contained higher level of natural antioxidant, especially tocopherols. In a study conducted by Lagunes-Galvez et al. (2002) on the oxidative stability of mayonnaise made from sunflower oil during storage and day light irradiation, no evidence of lipid oxidation was observed during 10 months storage at 20°C. The high content of tocopherols in the sunflower oil was mentioned as the reason for the good stability of the mayonnaise towards oxidation. As to the recommendation of these authors, once opened, mayonnaise should be in the refrigerator and be consumed within one month, because each opening of the jar provides new oxygen supply that enhances oxidation.

2.2 Fat Replacers

2.2.1 Definition

Fat replacers are ingredients designed to replace all or part of the fat in a food product which have certain desirable physical or sensory attributes of the fat, for example flavour, palatability, mouth feel, creaminess, etc., which they replace and have a potential of reducing the calorific value of the food product (Keeton, 1994).

An ideal fat replacer should be: (Štern & Hermann-Zaidins, 1992; Grossklaus, 1996; Cheung et al., 2002)

- Completely safe for consumption
- Of fewer calories than a similar product with naturally occurring fats
- Imitate tastes and looks of fats
- Free of any objectionable odour
- Heat resistant
- Biodegradable and should not place any additional burden on the environment
- Similar price to fat

2.2.2 Types of Fat Replacers

The terms and definitions used to describe fat replacers vary among authors and there are generally two categories of fat replacers: Fat Substitutes and Fat Mimetics.

1. Fat Substitutes

Fat substitutes are macromolecules that physically and chemically resemble conventional fats and oils (triglycerides) which can replace the fat in foods on a one-to-one, gram-for-gram basis, but have no calories (non-nutritive) or lower calories (nutritive) compared to the traditional fats (Jones, 1995). They are either chemically synthesized or derived from conventional fats and oils by enzymatic modification (Sandrou & Arvanitoyannis, 2000). Fat substitutes are often referred to as fat-based fat replacers. They are used as fat replacers because the chemical modification makes them non-metabolizable and/or unabsorbable by the body contributing no calories to the body (Gross-Man et al., 1994; Mattson and Nolen, 1972). Others, like medium chain triglycerides (MCT) can supply calories but are not stored in the body and instead immediately used up (expended) (Drake & Swanson, 1995).

2. Fat Mimetics

Fat mimetics are non-fat substances that imitate a particular function of fat and act as a partial replacer of fats (Anonymous, 1996). It cannot replace fat on a one-to-one, gram-for-gram basis. These materials are either proteins or carbohydrates and they are often known as protein-based fat replacers or carbohydrate-based fat replacers (Sandrou & Arvanitoyannis, 2000). Generally fat mimetics are not useful as a frying medium, because they mimic fat by entrapping water and are best used when the application has sufficient moisture.

i. Protein-Based Fat Replacers

Several fat replacers are derived from a variety of protein sources, including egg, milk, whey, soy, gelatin, and gluten. Some of these protein-based fat mimetics are microparticulated to form microscopic (0.1-2.0 μm in diameter) coagulated round deformable particles that mimic the mouthfeel and texture of fat (Singer & Moser, 1993). Others are processed to modify other aspects of ingredient functionality, such as water-binding and emulsification properties (Akoh, 1998). They are used as fat replacers because they act as microparticles to mimic the smooth mouthfeel of fats while contributing about 54% less calories compared to fats (Akoh, 1998; Lucca & Tepper, 1994).

ii. Carbohydrate-Based Fat Replacers

Carbohydrates have been used in some foods to partially or totally replace fat (Lucca & Tepper, 1994). Carbohydrate materials mostly used as fat replacers are starch and starch derivatives, and non starch polysaccharides such as gums, cellulose and dietary fibres. Carbohydrate-based fat replacers achieve fat replacement mainly because of their hydrocolloid nature by stabilizing substantial quantities of water in a gel like matrix, resulting in lubricant and flow properties similar to those of fats, and they function primarily to increase viscosity and body, and to provide a creamy, slippery mouth feel similar to that of fat (Yackel & Cox, 1992; Glicksman, 1991; Giese, 1996). They can achieve about 54% calorie reduction, and even more because of the water in their formulation, compared to fats. The major limitations of carbohydrate-based fat replacers are their high water content which could increase the water activity and their flavour masking effect (Yackel & Cox, 1992).

Table 2.1 summarizes the different types of fat replacers. It is noted that the oil replacement level is mostly up to 50% and carbohydrate-based fat replacers are most commonly used compared to protein-based and fat-based fat replacers.

2.2.3 Starch as Fat Replacer

Starches of varying sources, types, and functional properties can be used in fat replacing systems to provide sensory properties of oil, e.g. slippery mouthfeel (Akoh, 1998). Starch may be preferred as fat replacer than other types of materials due to its reasonable cost and wide availability (Lucca & Tepper, 1994).

2.2.3.1 Starch composition and structure

Starch is the major storage polysaccharide in foods of plant origin. It occurs in the form of semi-crystalline granules. Starch granules have size ranging from 1 µm to 100 µm in diameter (Thomas & Atwell, 1999). Maize starch (Figure 2.5) has spherical and angular granules (Whistler & Bemiller, 1997). Teff starch has polygonal-shaped granules (Figure 2.6), and the granules are found in groups forming compound granules compared to the single granules of maize starch (Bultosa et al., 2002). Oats and rice also have compound starch granules (Thomas & Atwell, 1999). Compared to maize starch granules (5 - 30 µm in diameter) (Thomas & Atwell, 1999), teff starch granules are smaller in size, 2 - 6 µm in diameter (Bultosa et al., 2002).

Table 2.1: Summary of the main categories of fat replacer

Category/type	Composition and energy density	Functional properties	Food applications	Remark	Ref.
Fat-based					
✓ Olestra	Sucrose polyester, 6 – 8 fatty acids esterified. (non-caloric)	Modifying texture and mouth feel	Savoury, snacks, crackers	It can fully replace fats in snacks and crackers, but may have side effect on the gastrointestinal tract like loose stool.	1, 2
Protein-based					
✓ Simplesse®	Microparticulated protein from egg or milk (0.04 – 0.17 KJ/g)	Act as microparticles, modify mouth feel	Salad dressings, cheese, ice cream	It can replace up to 50% of the fat in cheese, and salad dressings. It may have flavour masking effect. Allergens (milk and egg proteins) and cultural issues (animal proteins) could also be other draw backs.	3, 4, 5
✓ Dairy-Lo®	Modified whey protein concentrate (0.04 – 0.17 KJ/g)	Act as microparticles, bind water, act as emulsifier	Cheese, yoghurt, ice cream, salad dressings	It can replace up to 50% of the fat in cheese, and salad dressings. It may have a draw back of allergens and cultural issues like simplesse®	3, 4, 5
Carbohydrate-based					
✓ β-glucan	Soluble fibre extracted from oats and barley (0.04 – 0.17 KJ/g)	Add body and texture	Sausages, burgers, mayonnaise, yoghurt	It can replace up to 75% of the fat in mayonnaise and yoghurt. It may affect the colour and appearance negatively	6, 7
✓ Gums and pectin	Zanthan, guar, locust bean, carrageenan, alginates, fruits (non-caloric)	Retain moisture, modify texture and mouth feel	Mayonnaise, cheese, yoghurt	Can replace up to 50% of the fat in mayonnaise and cheese.	3, 7, 8, 9, 10
✓ Modified cellulose	Various plant sources in microcrystalline form (non-caloric)	Modify texture and mouth feel	Salad dressings, confectionary, dairy products		2, 9, 11
✓ Modified starches	Variety of starch sources (0.04 – 0.17 KJ/g)	Modify texture, gelling, thickening, and stabilizing	Mayonnaise, processed meats, yoghurt, cake	Can replace up to 50% of the fat in mayonnaise, yoghurt and sausages. It may mask flavours and may have short shelf-life because of high water content	9, 12, 13

References: 1- Akoh (1998), 2- Sandrou and Arvanitoyannis (2000), 3- Lobato-Calleros et al. (2001), 4- Sandoval-Castilla et al. (2004), 5- Gillat and Lee (1991), 6- Worrasinchai et al. (2006), 7- Brennan and Tudorica (2008), 8- Liu et al. (2007), 9- Wylie-Rosett (2002), 10- Su et al. (2010), 11- Glicksman (1991), 12- Mun et al. (2009), 13- Aime et al. (2001)

Jane (1992) has proposed small-granule starches having a granule diameter similar to lipid micelles might have a potential as fat replacer.

Models have been developed to explain starch granule structure, one of the probable models being the "cluster" or "Blocklet" model (Gallant *et al.*, 1997; Ohtani et al., 2000). Figure 2.7 (A-D) illustrates the theory of the "blocklet" model. Figure 2.7A shows the alternating crystalline (hard shell) and semi-crystalline (soft shell) layers of the granule at the lowest level of granule organization. The alternating semi-crystalline and crystalline rings (Figure 2.7B) are proposed to be due to the assembly of two or three layers of blocklets (Gallant et al., 1997). Each blocklet contains alternating crystalline and amorphous lamellae (Figure 2.7C). Figure 2.7D shows the alternating crystalline and amorphous lamellae of the blocklet. The crystalline lamellae of amylopectin (Gallant et al., 1997) double helix clusters alternate with amorphous lamellae made up of amylopectin branching regions (Gallant *et al.*, 1997) and contains free amylose and amylose complexed with lipids (Gunaratne & Corke, 2004).

The starch granule is primarily composed of two α-glucose polymers, amylose and amylopectin (Cura et al., 1995). Amylose is a mostly linear polymer of α-D-glucopyranosyl units linked via α (1-4) glycosidic linkage (Lui, 2005). Amylose has a molecular weight of $1.6 \times 10^5 - 7.1 \times 10^5$ (Lui, 2005). Teff has amylose with average molar mass in the range of $1.5 \times 10^7 - 3.0 \times 10^7$ g/mol compared to 2.3×10^7 g/mol for maize (Bultosa et al., 2008).

Although illustrated as straight chain for the sake of simplicity, amylose often forms a helical structure (Thomas & Atwell, 1999). Amylose forms two types of helices; single and double helix (Figure 2.8). The single helix contains six glucose residues per turn and has a characteristic hydrophobic core, due to the presence of many CH groups, and hydrophilic exterior due to the presence of many hydroxyl groups (Zobel, 1988). Amylose molecules form complexes with guest molecules such as fatty acids, mono and diglycerides, iodine, flavour compounds, due to the interaction between the hydrophobic interior of the amylose and these guest molecules (Jackson, 2003; Godet et al., 1995). The amylose double helix is formed when an amylose molecule interacts with another, stabilized by hydrogen bonds and Van der Waals forces (Oates, 1997). Amylose double helices are partially responsible for starch retrogradation (Gudmundsson, 1994).

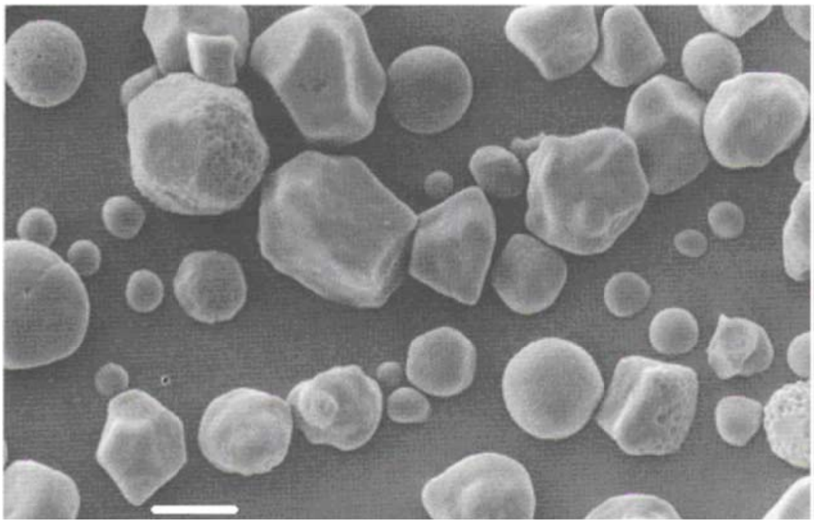


Figure 2.5: Spherical and angular maize starch granules (Scale bar = 10 µm) (Whistler & BeMiller, 1997).

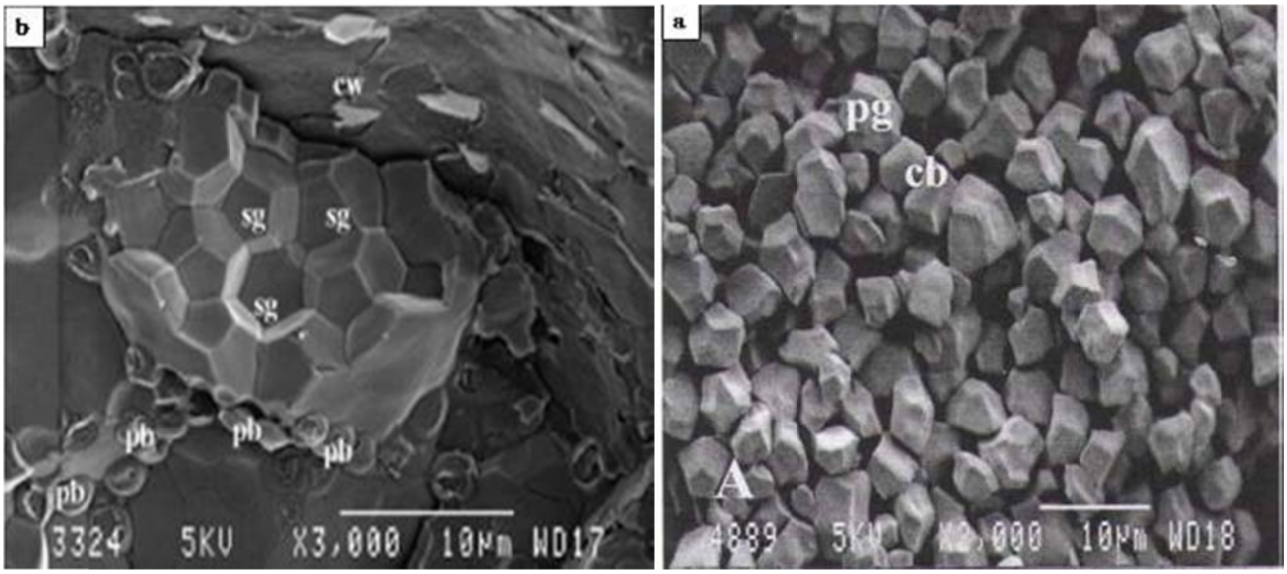


Figure 2.6: a) Individual teff starch granules, where pg = polygonal granules and cb = cubic granules. b) The compound teff starch granule as found in the endosperm of the teff grain; where sg = individual starch granules, pb = protein bodies and cw = cell wall (Bultosa et al., 2002).

Amylopectin is a highly branched polymer of α-D-glucopyranosyl units linked via α (1-4) glycosidic linkages to form a linear chain, and α (1-6) glycosidic linkages at branch points (Parker & Ring, 2001). The basic organization of the chains is described in terms of the A, B, and C chains (Figure 2.9). The A chains are the shortest outer chains with no branches attached to them. They are attached to an inner chain (chain B) at their potential reducing ends by α (1-6) glycosidic bond. The B chains are those which bear other chains (chain A) as branches. The C chain is the single chain which carries the sole reducing group while it carries other chains as branches (B chains) (Buleon et al., 1998; Zobel, 1988; Lui, 2005; Parker & Ring, 2001).

Amylopectin is a much larger molecule compared to amylose with a molecular weight ranging ca. $1.0 \times 10^7 – 5.0 \times 10^8$ (Whistler & BeMiller, 1997). Bultosa et al. (2008) found the average molar mass of teff amylopectin to be in the range of $10.1 \times 10^7 – 10.5 \times 10^7$ g/mol, compared to an average of 19.6×10^7 g/mol for maize.

As reviewed by Zobel (1988) and Kent and Evers (1994), starches produce four distinct patterns when exposed to X-rays (Figure 2.10). These patterns are: A, B, C and V. The A type (Imberty et al., 1988) which is found in most cereal starches consists of six bundles of double helices packed into a monoclinic array. The B type (Wu & Sarko, 1978; Imberty & Perez, 1988) which is found in some tuber starches and high amylose cereal starches is a more highly hydrated and open structure, consisting of double helices in a hexagonal array. The C type which is found in legume starches and starches from other tropical tubers, is thought to be a combination of the A and B type pattern (Gallant et al., 1997). When amylose forms a complex with lipids it forms a V type diffraction pattern (Godet et al., 1993).

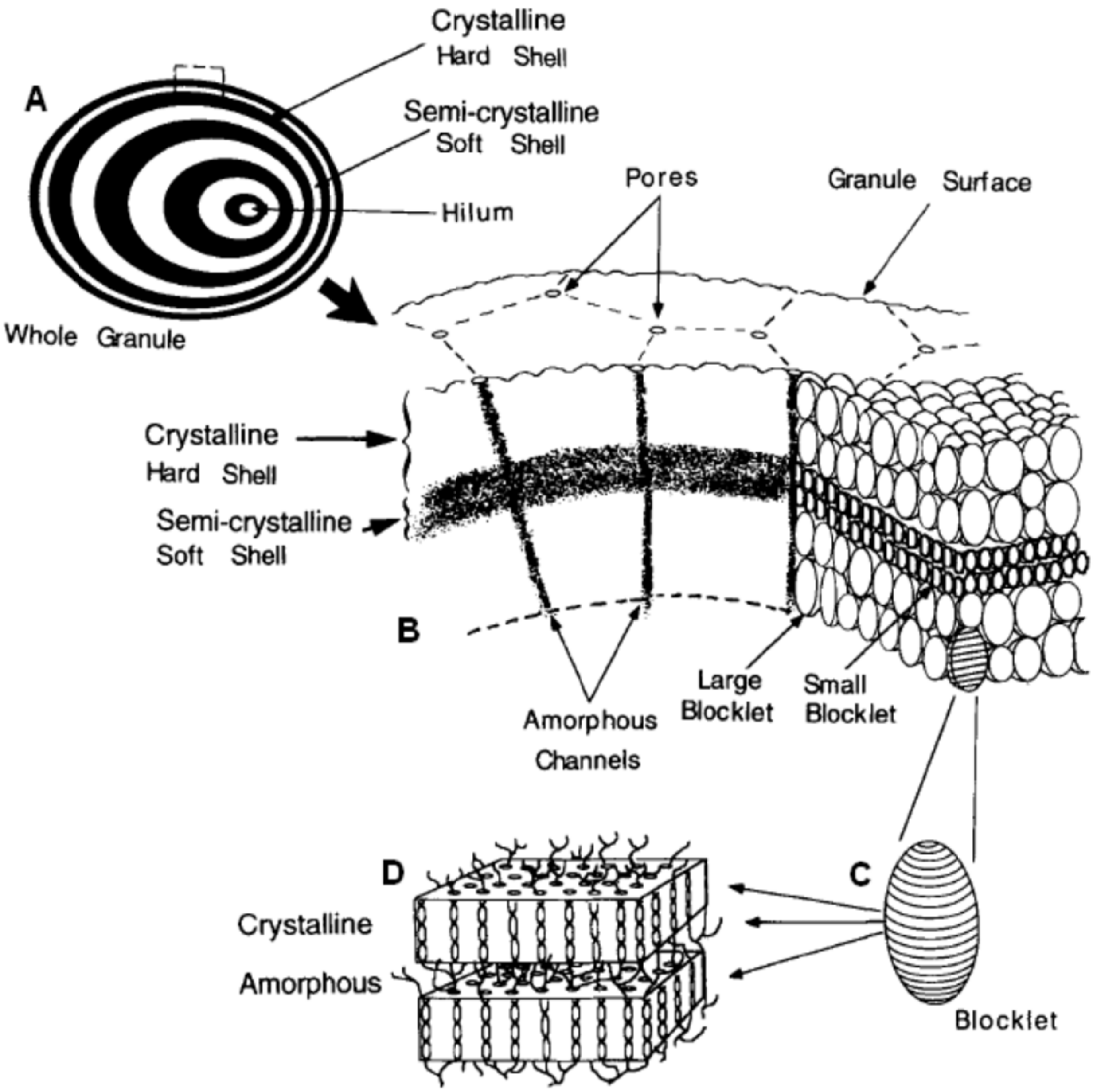


Figure 2.7: Overview of starch granule structure. At the lowest level of granule organization (upper left), the alternating crystalline (hard) and semi-crystalline (soft) shells are shown (dark and light colours, respectively). The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the hilum is shown off centre. At a higher level of structure the blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semicrystalline shells than in the crystalline shells. At the next highest level of structure one blocklet is shown containing several amorphous crystalline lamellae (Gallant et al., 1997).

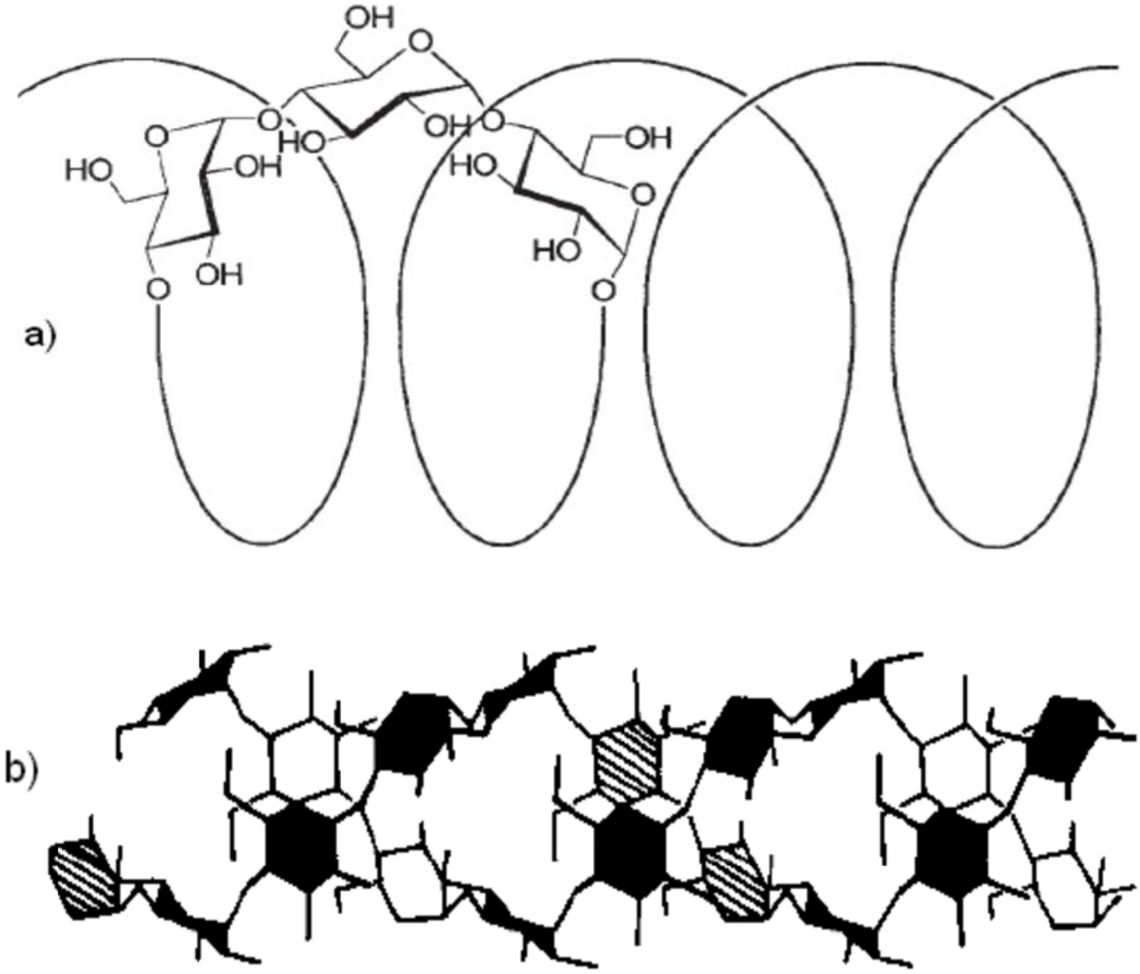


Figure 2.8: (a) The amorphous amylose single helix (Immel & Lichtenhaler, 2000) and (b) the crystalline amylose double helix (Zobel, 1988).

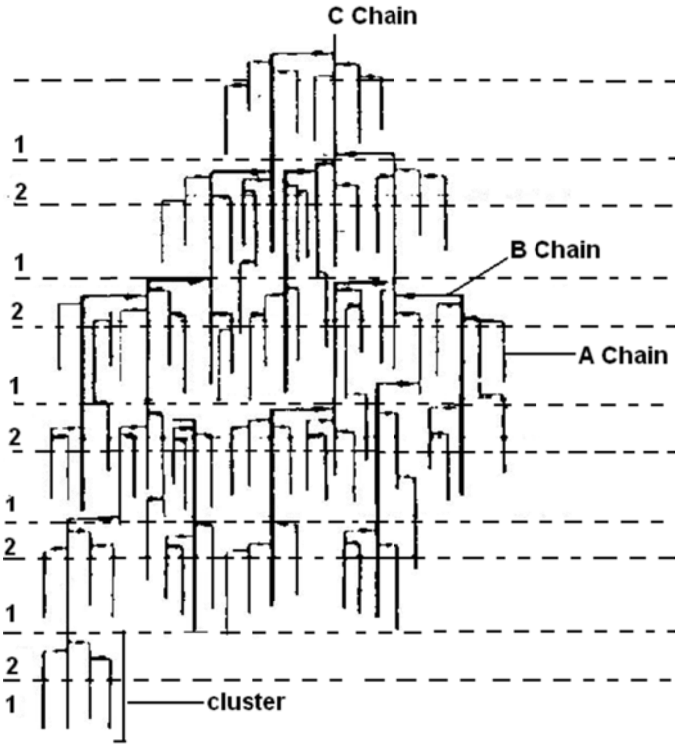
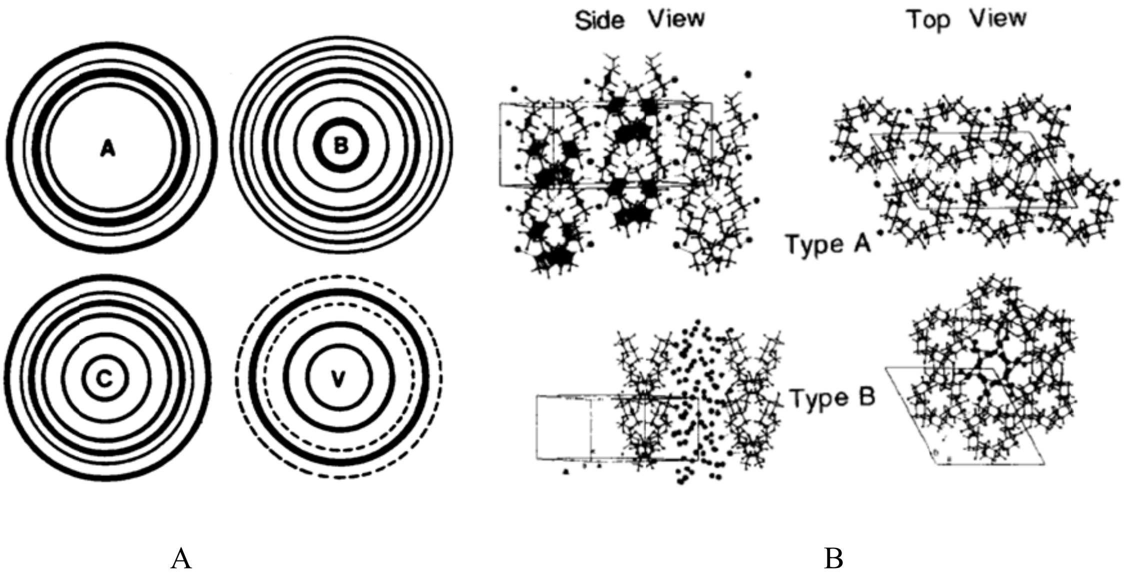


Figure 2.9: Schematic representation of the A, B and C chains and clusters of amylopectin. Bands marked '1' are considered crystalline and '2,' amorphous (Kent & Evers, 1994).



A

B

Figure 2.10: A) Starch X-ray pattern designations (Zobel, 1988) B) The crystal structures of A and B-type starches (Gallant et al., 1997).

2.2.3.2 Starch gelatinisation, pasting and retrogradation

Native starch granules typically exhibit a limited amount of swelling in cold water. However, when prepared as an aqueous slurry and heated, the granules hydrate and swell to several times their initial size (Atkin et al., 1998; Parker & Ring, 2001). In the course of this process at a fairly well defined temperature (start of gelatinisation temperature) the starch granules start to lose their birefringence and molecular order. This is due to the disruption of hydrogen bonds and this occurs initially in the amorphous region, because of weaker hydrogen bonds in these areas (Singh et al., 2003). Complete loss of molecular order within a starch granule occurs over a range of temperature, which is characteristic of a particular starch (Parker & Ring, 2001; Jackson, 2003). For teff starches, this gelatinisation temperature range is 68 °C – 80 °C (Bultosa et al., 2002). Maize starch gelatinizes between 62 °C - 80 °C (Whistler & BeMiller, 1997).

As the temperature increases the starch granules continue to swell as they absorb more water, causing an increase in viscosity in the course of time and a peak viscosity occurs when largest percentage of swollen granules are present (Thomas & Atwell, 1999). After the peak viscosity with continued heating and shear, the viscosity of the starch paste drops quickly due to granular disintegration and leaching out of solubilised starch polymers from the swollen granules to the solution. The polymers can also align themselves in the direction of the shear to further reduce the viscosity (Whistler & BeMiller, 1997). Bultosa and Taylor (2004) found that teff starch had a greater amount of amylose leaching with time than maize starch.

Upon cooling the viscosity again starts to rise. The increase in viscosity during the cooling period indicates a tendency of various constituents present in the hot paste to re-associate or retrograde as the temperature of the paste decreases (Singh et al., 2003). Amylose molecules undergo strong retrogradation because of their predominantly linear structure (Parker & Ring, 2001). Amylopectin shows slow and weak retrograding tendencies due to its highly branched structure. As the starch retrogrades the water may be squeezed out and syneresis may occur. Retrogradation is regarded as a limitation of native starch for certain food applications, for example the staling of bread (Karim et al., 2000).

2.2.3.3 Starch Modification

Although native starch can sometimes be used as fat replacer, starches are commonly modified through physical and/or chemical techniques to be used as fat replacers by enhancing the stability, creaminess and moisture retention of food products (Jane, 1992; Akoh, 1998). Chemical modifications such as cross linking, stabilization, acid modification and oxidation have the greatest effect on starch functionality (BeMiller, 2003). Depending on the chemicals used chemical modification can result in starches with slower retrogradation, reduced gelling tendency, reduced syneresis and improved film formation (Bao et al., 2004). On the other hand chemical modification (the addition of chemicals) has raised health concerns especially for food application, and there is a demand to use naturally occurring compounds to modify starches. Fatty acids are one group of naturally occurring compounds which are able to interact with the hydrophobic interior of the amylose helix to form inclusion complexes (Jackson, 2003; Raphaelides & Karkalas, 1988).

Fatty acids belong to a group of lipids which consist of open-chain compounds possessing polar heads and non-polar tails (Campbell & Farrell, 2003). Most common fatty acids have chain lengths of 12 – 22 carbon units (Christie, 2003). Stearic acid (Figure 2.11) is a completely saturated 18 carbon unit fatty acid. With the carboxyl group serving as the hydrophilic head and the hydrocarbon chain making up the hydrophobic tail, fatty acids react with the hydrophobic core of the amylose helix (Raphaelides & Karkalas, 1988).

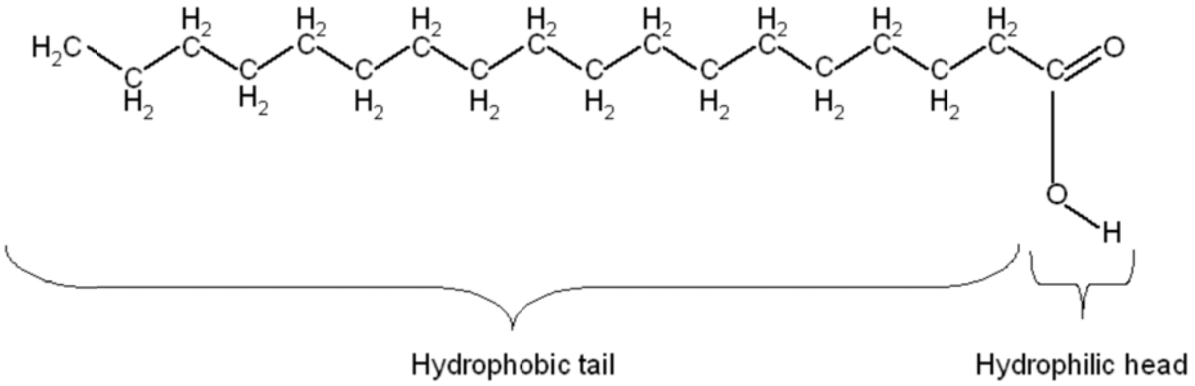


Figure 2.11: The structure of stearic acid showing the hydrophilic carboxyl group (head) and the hydrophobic carbon chain (tail) (D'Silva, 2009).

D'Silva (2009) used stearic acid to modify teff and maize starch and subjected the starch to extended pasting. The stearic acid was found to influence both teff and maize starches in the following way:

When held at 91 ^{o}C for 2 hr a second viscosity peak was observed during the holding time (Figure 2.12). This second viscosity peak was first observed for maize by Nelles et al. (2000) and later by Bajner (2002) when native maize starch was subjected to an extended pasting profile (approximately 110 min. at 91 ^{o}C). The second peak was not observed when the lipid fraction of the starch was removed (Nelles et al., 2000) showing that the second viscosity peak could be due to the formation of amylose-lipid complex. When D'Silva (2009) increased the amount of stearic acid added to maize and teff starches the magnitude of the second viscosity peak increased. Bajner (2002) also found an increase in the second viscosity peak with increasing stearic acid concentration, indicating the second viscosity peak could be due to amylose-lipid complex formation. Burger (2010) also found X-ray difractograms of teff and maize starches modified with stearic acid (0.25% and 1.5%) to have the characteristic V-type amylose-lipid complex crystallinity peaks. DSC thermograms also showed melting of crystallites typical of amylose-lipid complexes at 99-120 ^{o}C (Burger, 2010). This also indicates the second viscosity peak could be due to amylose-lipid complex. Generally, amylose-lipid complex formation occurs in starch containing natural lipids or with the addition of lipids to defatted starch (Godet et al., 1993). The complex (Figure 2.13) is formed when the hydrophobic tail occupies the central hydrophobic core of the amylose helix consisting of six, seven, or eight glucose units per turn (Karkalas & Raphaelides, 1986) stabilized through hydrophobic interactions and Van der Waals forces (Raphelides & Karkalas, 1988). The head of the fatty acid lies outside the amylose helix because the steric hindrance and electrostatic repulsions prevent the carboxylic head from entering the helix (Godet et al., 1993). As reviewed by Putseys et al. (2010) 18 – 24 glucose units are required for the complexation with one lipid molecule (fatty acid or monoacyl glycerol) with a tail of 14 – 18 carbon atoms.

Teff and maize starch pastes subjected to extended pasting with the addition of 1.5% stearic acid were found to be non-gelling (D'Silva, 2009). High concentration of emulsifier (mixture of a polyglycerol ester and a monoglyceride) were also found to prevent potato amylose gels from forming, instead thick opaque pastes were formed (Richardson et al., 2004).

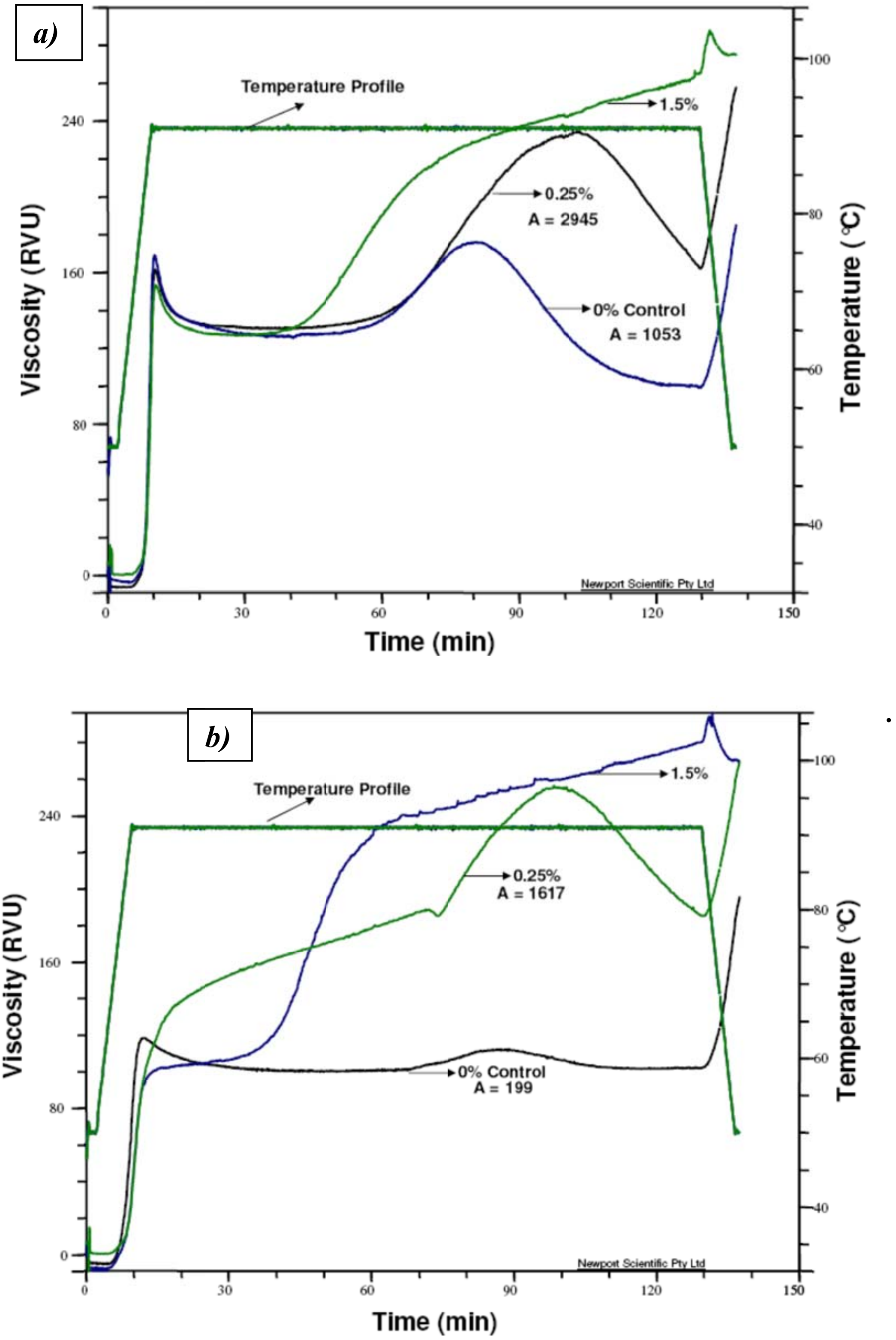


Figure 2.12: Effect of stearic acid concentration on the pasting properties of teff starch for the extended pasting cycle (2 h holding at 91 °C), where A represents the relative area under the peak. A) maize starch b) teff starch (D'Silva, 2009).

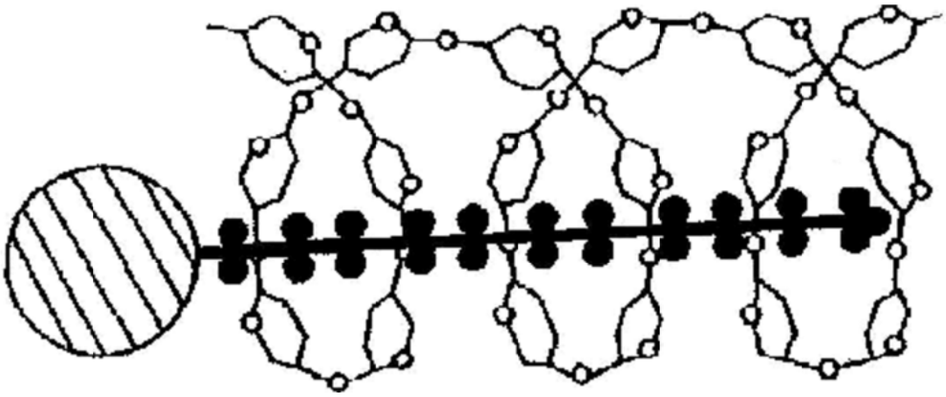


Figure 2.13: The amylose-monostearin complex showing the carbon chain of the fatty acid inside the helical space, while the head lies outside the helix (Carlson & Larsson, 1979).

Table 2.2: The effect of 1.5% stearic acid addition on the paste properties of maize and teff starch pastes.

Effect of added stearic acid on:	**Maize starch pastes**	**Teff starch pastes**
Viscosity of the first peak	Decreased	Viscosity increased steadily but did not form a peak.
Viscosity of the second peak	Increased	Increased
Final viscosity	Increased	Increased
Gel Texture	Reduced gelling ability	Reduced gelling ability
X-ray pattern	Indicate formation of V-type pattern	Indicate formation of V-type pattern

*Adapted from D'Silva (2009)

Some proposed reasons for the effect of fatty acids on the starch gelling ability are (Gudmundsson, 1994):

1) The amylose-lipid complex interferes with amylopectin re-crystallization, thus retarding retrogradation.

2) The amylose-lipid complex interferes with retrogradation by retarding water distribution.

3) Co-crystallization of amylose with other compounds reduces the ability of amylose to form double helices that are characteristic of retrograded starches.

The soft texture (non-gelling behaviour) and the high viscosity of teff and maize starch pastes modified with stearic acid indicates that these may have a potential to be used as fat replacers in low-calorie foods.

2.2.3.4 Modified starches as fat replacer in mayonnaise and other products

Starches modified with different physical and chemical methods have been used as fat replacers in mayonnaise. However, there is limited research on the use of lipid-modiifed starches as fat replacer in food systems. Ma et al. (2006) used dextrozyme to degrade maize starch into fine particles and used it as fat mimetics in low fat mayonnaise. Their results showed that 60% fat reduced mayonnaise with hydrolyzed maize starch had similar sensory quality to full fat mayonnaise. Mun *et al.*, (2009) used 4-α-glucanotransferase (4αGTase) enzyme modified rice starch as fat replacer in mayonnaise combined with xanthan gum and found it a useful fat replacer in a 50% reduced fat mayonnaise. Starch-lipid complexes were used in yoghurt as additives (Singh & Kim, 2009) or to replace milk solids (Singh & Byars, 2009) and in soft-serve ice cream as fat replacer (Byars, 2003). However, use of starch-lipid composites in mayonnaise has not been reported.

2.3 Concluding Remarks

- Mayonnaise is a high fat food with 70 – 80% oil and the consumption of high fat foods is related with lifestyle diseases like obesity, cancer and cardiovascular diseases.
- Thus food scientists have developed fat replacers as a solution to produce low fat/calorie food products commonly up to 50% replacement.
- Starch, the most available biopolymer, is used as fat replacer in mayonnaise and salad dressings. Although native starch can be used as fat replacer, starches are commonly modified by chemical/physical methods to be used as fat replacer.
- Chemical modification of starch (the use of chemicals) has raised health concerns. Therefore, there is a demand to use naturally occurring compounds like fatty acids for starch modification.
- Recently D'Silva (2009) used stearic acid (naturally occurring fatty acid) to modify teff and maize starches. The addition of stearic acid to teff and maize starches produces pastes with higher viscosity and pastes with lower gelling capacity compared to the control starch pastes.

- These higher viscosity and lower gelling properties of the teff and maize starch pastes present an opportunity for these to be used as fat replacers.

3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

3.1.1 Low calorie mayonnaise type emulsions (LCMTE) with modified teff and maize starches with stearic acid will have more similar flow and viscoelastic properties to full fat mayonnaise than LCMTE with unmodified teff and maize starch. The possible reason for this could be the non-gelling behaviour of modified teff and maize starches with stearic acid (D'Silva, 2009). The unmodified teff and maize starch pastes will retrograde and form a gel in the LCMTE stored at room temperature. The gelling behaviour of the unmodified starches will result in non-pourable LCMTE and those LCMTE will have different flow and viscoelastic properties compared to the full fat mayonnaise. On the other hand modified teff and maize starches with stearic acid will not form a gel possibly due to the formation of amylose-lipid complexes which could inhibit the reassociation of two amylose molecules and interfere with the crystallization of amylopectin and retards retrogradation (Gudmandsson, 1994). The non gelling behaviour of modified teff and maize starches with stearic acid will result in a pourable LCMTE and will have similar flow and viscoelastic properties to full fat mayonnaise.

3.1.2 The microstructure of LCMTE with modified teff and maize starches will be more similar to full fat mayonnaise compared to LCMTE with unmodified teff and maize starches. LCMTE with unmodified teff and maize starches will have smaller oil droplets (related to higher viscosity) compared to full fat mayonnaise and LCMTE with modified teff and maize starches with stearic acid. This is due to the gelling behaviour of the unmodified teff and maize starches which will hold the small oil droplets, formed during homogenization apart from each other. On the other hand due to the non-gelling behaviour of the modified teff and maize starches with stearic acid (D'Silva, 2009) some of the oil droplets may move and interact with each other which may lead to coalescence of some oil droplets resulting in relatively larger oil droplets similar to those in the full fat mayonnaise.

3.2 Objectives

3.2.1 To determine the effect of teff and maize starches modified with stearic acid on the flow and viscoelastic properties of LCMTE compared to unmodified teff and maize starches.

3.2.2 To determine the effect of teff and maize starches modified with stearic acid on the microstructure of LCMTE compared to unmodified teff and maize starches using optical and confocal laser scanning microscopy.

3.2.3 To determine the effect of teff and maize starches modified with stearic acid on the freeze-thaw and high temperature stability of LCMTE compared to unmodified teff and maize starches.

4. RESEARCH

Rheology and microscopy of low calorie mayonnaise-type emulsions made with stearic acid- modified maize and teff starches

ABSTRACT

Modification of teff and maize starches with stearic acid, a naturally occurring fatty acid, has resulted in a paste with reduced gelling ability and higher viscosity. These modified teff and maize starches were proposed as potential fat replacers. The objective of this study was to determine the effect of modified teff and maize starches on the rheological properties, microstructure and freeze-thaw and high temperature storage stability of low-calorie mayonnaise type emulsion (LCMTE). Starch suspensions (10% w/v of starch in water) containing stearic acid (1.5%) were pasted for an extended holding time of 2 hr at 91 °C in a Rapid Visco Analyser (RVA) and used to prepare LCMTE with the oil replaced at 50%, 80% and 100% in comparison to a full fat mayonnaise.

Flow property measurements showed a decrease in yield stress and viscosity values of LCMTE when using modified teff and maize starches to replace oil compared to LCMTE with unmodified teff and maize starches. Increasing level of oil replacement (from 50% to 100%) increased the viscosity and LCMTE with maize starch were found to have higher yield stress and viscosity values than LCMTE with teff starch. All samples were found to be shear thinning with $n<1$. Optical and confocal laser scanning microscopy showed LCMTE with modified starches to have large size oil droplets compared to LCMTE with unmodified starches. The smaller size oil droplets of LCMTE with unmodified starches are related to the higher yield stress and viscosity values compared to the LCMTE with modified starches which have larger size oil droplets. LCMTE with maize starch were found to have smaller size oil droplets than LCMTE with teff starch. All mayonnaise samples were found to be more solid like with storage modules G' to be greater than loss modules G" and $\tan\delta < 1$. All the LCMTE were found to be more stable to freeze-thaw cycles and high temperature storage. At 50% oil replacement unmodified and modified teff and maize starch with stearic acid could produce LCMTE. When the oil content was further decreased to 80% and 100% only the LCMTE with modified starches were similar to the full fat.

4.1 Introduction

Production of low-calorie foods has been developed due to the positive relation between lifestyle diseases such as diabetes and cardiovascular diseases and consumption of high fat foods (Van Gaal et al., 2006; Van Dam et al., 2002; & Hu et al., 1997). The design of low calorie food necessitates the use of fat replacers to mimic some of the functional properties of fat. Otherwise the low calorie foods will have inferior sensory properties. Mayonnaise is considered as a high calorie food as it contains over 70% oil (Depree & Savage, 2001; Su et al., 2010). It is highly used as a sauce and condiment in various dishes and in most fast food outlets.

Starch, a natural biopolymer, can be used as fat replacer. Teff [*Eragrostis tef* (Zucc.) Trotter], a highly underutilized tropical crop indigenous to Ethiopia, is a potential cereal source of starch. Teff starch has small granules (2-6 μm) and slightly different properties compared to other tropical cereals (e.g. maize) and has been suggested to be used as fat mimetics (Bultosa et al., 2002). Even though native starch can sometimes be used as fat replacer, it is usually modified by physical or chemical methods to be used as fat replacer. This is because native starch has some limitations for food use like low thermal resistance, high tendency towards retrogradation and rubbery weak bodied gels (Singh et al., 2007; BeMiller, 2003). Chemical and physical modification of starch can improve its properties such as thickening, binding, mouthfeel, gelling, dispersion or cloud formation (Singh et al., 2007). Stearic acid has been found to modify maize (Bajner, 2002) and teff starch pasting properties (D’Silva, 2009 and Burger 2010). Stearic acid added to a level of about 1.5% can increase the pasting viscosity twice during pasting for more than 1 hr. D’Silva (2009) also found teff and maize starch pastes modified with stearic acid to have a non-gelling behaviour compared to the gelling behaviour of the unmodified starches. Both D’Silva (2009) and Burger (2010) proposed that the modified teff starch with stearic acid can be used as fat replacer in high fat foods because of its non-gelling behaviour and increased viscosity. No research has been conducted to test this proposition.

The objective of this study was to determine the effect of teff starch modified with stearic acid on the rheological properties and microstructure of low-calorie mayonnaise type emulsion.

4.2 Materials and Methods

4.2.1 Samples

A white teff variety (Witkop) was obtained from PANNAR, Kroonstad, South Africa. Commercial maize starch, Amyral (from a white maize variety) was obtained from Tongaat Hulett®, Edenvale, South Africa. Pasteurized and spray dried Egg yolk powder, product code YLKPW 500, was obtained from Ovipro (PTY) Ltd., Bronkhorstpruit, South Africa. Stearic acid was obtained from Sigma-Aldrich (product code S 4751 – 25 G). Sunflower oil, white spirit vinegar, salt and sugar were bought from a local supermarket. All chemicals other than those bought from local supermarket were of analytical grade unless specified otherwise.

4.2.2 Starch extraction

Teff starch was extracted by the methods of D'Silva (2009) and Bultosa et al. (2002). Teff grain was sieved to remove impurities and the grain was milled in a laboratory hammer mill to pass through an 800 µm screen. The flour was defatted with hexane. One part flour was added to 3 parts hexane and stirred at 25 ^{o}C for 1 h. This was repeated three times. Defatted flour was suspended in distilled water in a 1 part flour: 5 part distilled water (wt: vol.) ratio for one hour at 25 ^{o}C. The flour suspension (slurry) was then wet milled with a Retsch Mill ZM 200 (Haan, Germany) and passed through a 250 µm screen. The liquid that passed the screen was retained and filtered with 75 µm and 38 µm hand sieves sequentially. The residues (mostly fibre) on the wet mill screen and the hand sieves were discarded. The filtrate was centrifuged at 8000 rpm for 10 min at 25 ^{o}C, the supernatant was decanted off and the brown protein layer was scraped off. The remaining starch pellet was resuspended in distilled water and centrifuged, the supernatant was decanted off and the brown layer was scrapped off. This procedure was repeated until a white starch pellet was obtained. The white starch pellets were then freeze dried and proximate analysis was done to check the purity of the starch.

4.2.3 Proximate analyses

The AACC methods 44-15A, 08-01, 30-12A, and 46-30 (American Association of Cereal Chemists, 2000) were used to determine the moisture, ash, crude fat, and protein contents respectively of teff and commercial maize starches. The Megazyme total starch assay kit

(Megazyme® International Bray, Ireland) was used to determine the percentage composition of total starch. This method uses thermostable α-amylase and amyloglucosidase to hydrolyse starch to glucose. Glucose is then quantified calorimetrically by the glucose oxidase-peroxidase reaction.

4.2.4 Incorporation of stearic acid into starch

Stearic acid (1.5% on the weight basis of dry starch) was dissolved in ethanol in a beaker. Teff or maize starch was then added to the solution. The starch: ethanol ratio was 1: 3 (wt: vol.). The beaker was then covered with Parafilm and foil and placed in a shaking water bath at 50 °C for 30 min. The ethanol was then evaporated off in a force draught oven at 40 °C. The control starch samples were treated exactly the same, but without the addition of stearic acid.

4.2.5 Starch pasting (extended holding time)

Rapid Visco Analyser (RVA model 3D) (Newport Scientific, Warriewood, Australia) was used for pasting. Starch (2.8 g dry basis) was suspended in distilled water to get a total of 28 g starch-water suspension. The pasting condition used was: initial stirring at 960 rpm at 50 ^{o}C for 30 s and thereafter at 160 rpm for the entire period. The temperature was stabilized at 50 ^{o}C for 2 min and was increased from 50 ^{o}C to 91 ^{o}C at a rate of 5.5 ^{o}C/min, and then held at 91 ^{o}C for 1 h 51 min. The total time for the whole process was 2 h. The paste was readjusted to the original weight (28 g) with distilled water and used to replace oil in low-calorie mayonnaise type emulsions (LCMTE).

4.2.6 Mayonnaise preparation

The aqueous phase (vinegar, egg yolk, salt and sugar and water or starch paste for low-calorie mayonnaise type emulsions) was mixed first and oil was added little by little while continuously homogenising it at 8000 rpm using an Ultra Turrax T25 (JANKE & KUNKEL IKA® Labortechnik) for 5 min at room temperature. The mayonnaise formulations were similar to Ma et al. (2006), where only the percentage of the ingredients were adjusted as in Table 1 because of the level of oil replacement. In the low-calorie mayonnaise type emulsions the oil was replaced at 50, 80, and 100% levels. The mayonnaise samples were allowed to settle for 30 min before analysis.

Table 4.1: Formulations of full fat mayonnaise and low-calorie mayonnaise type emulsion samples (wt %)

	Full fat	**Low-calorie without fat replacer**	**Low-calorie 50% oil replacement**	**Low-calorie 80% oil replacement**	**Low-calorie 100% oil replacement**
Sunflower oil	70	35	35	14	-
Egg yolk	12	12	12	12	12
Vinegar	15	15	15	15	15
Salt	2	2	2	2	2
Sugar	1	1	1	1	1
Water	-	35	-	-	-
Starch pastes*	-	-	35	56	70

*Teff and commercial maize starch pastes, unmodified and modified with 1.5% stearic acid pasted for 120 min.

'-' indicates 0% of the ingredient was added

4.2.7 Flow Property measurement

Steady shear flow measurements were conducted with a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, German) using a bob and cup method. Plate-plate configuration at different gaps (1 and 1.5 mm) and vane methods were also used to determine the occurrence of wall slip (the wall slip will be discussed in the general discussion). The bob was inserted into the cup containing the mayonnaise samples and allowed to equilibrate for 30 min at 25 °C. Shear rate was increased from; 0.01 s^{-1} to 1000 s^{-1} and reduced back from 1000 s^{-1} to 0.01 s^{-1}. The measurements were taken at 25 °C. The experimental data were fitted to Herschel-Bulkley and Power Law models:

$\sigma = \sigma_0 + K(\dot{\gamma})^n$ (Herschel-Bulkley model), $\sigma = K(\dot{\gamma})^n$ (Power Law model)

Where σ_0 is the yield stress, σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (1/s), K is the consistency index ($Pa.s^n$) which is an indication of the viscosity, and n is the flow index where n=1 for Newtonian fluid, n<1 for shear thinning and n>1 for shear thickening materials.

4.2.8 Viscoelastic behaviour measurement

The viscoelastic behaviours of the mayonnaise samples were measured using a Physica MCR 301 Rheometer (Anton Paar, Ostfildern, German) by parallel plate method with a diameter of 50 mm at a gap distance of 1 mm at 25 °C. The linear viscoelastic range was determined with a strain amplitude sweep (0.1 – 100%) at constant angular frequency of 10 rad/s. Then frequency sweep was conducted by applying a constant strain of 0.5% which is within the linear viscoelastic range, over an angular frequency range of 0.1 – 100 rad/s.

After the loading step any excess sample volume protruding from the edge of the sensor was trimmed off with a spatula and the sample was allowed to rest for 10 min before analysis.

4.2.9 Confocal Laser Scanning Microscopy (CLSM)

Nile Red (Sigma Aldrich) was used to stain the oil phase and Nile blue (BDH Chemicals ltd, Poole, England) was used to stain the aqueous phase. Mayonnaise samples were prepared as described above in Section 4.2.6, but this time a 0.01% (w/v) aliquot of Nile Red solution was dissolved in the oil, and 0.01% (w/v) of Nile blue solution in the vinegar before the mayonnaise preparation.

From each sample a small amount of mayonnaise was placed on a concave microscope slide, covered with cover slip and analyzed on a Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany) at 40X magnification. Plane neoflar 100 x and Numerical aperture (N.A) 1.4 was used. The pixel time for both tracks 1 and 2 was 12.8 µs. Picture size was 512 x 512 pixels. The excitation and emission spectra for Nile Red were 488 nm and 550 – 603 nm respectively and for Nile blue 633 nm and 668 – 753 nm.

4.2.10 Optical Microscopy

From each sample, a drop of mayonnaise was placed on a microscope slide, covered with a cover slip and viewed using a Nikon Optiphot compound microscope (Nikon, Tokyo, Japan) with a 40 x numerical aperture objective lens. The pictures were taken with a Nikon DXM 1200 digital camera (Nikon, Tokyo, Japan) with Nikon ACT software (Nikon, Tokyo, Japan).

4.2.11 Freeze thaw stability

Forty grams of each sample was weighed into 100 ml plastic centrifuge tubes. The mayonnaise samples were centrifuged at 500 rpm for 30 min. The amount of separated liquid was weighed. The samples were then frozen at -18 ^{o}C for 24 h then thawed at 30 ^{o}C for 1 h and centrifuged at 500 rpm for 30 min. The amount of liquid separated was weighed. This procedure was repeated for ten consecutive cycles and freeze thaw stability at each cycle was calculated as:

$$\frac{\text{(Original weight)} - \text{(Weight of separated liquid)}}{\text{Original weight}} \text{X100}$$

Accelerated shelf life study was the rational of this experiment.

4.2.12 High Temperature Stability

Each mayonnaise sample was weighed six times into six eppendorf tubes with 2 g in each tube and stored at 50 ^{o}C for 0 h, 24 h, 7 days, 14 days, 21 days, and 28 days. Each time the samples were centrifuged at 3000 rpm for 10 min. The amount of liquid separated was weighed and the high temperature stability was calculated as:

$$\frac{\text{(Original weight)} - \text{(Weight of separated liquid)}}{\text{Original weight}} \quad \text{X 100}$$

Accelerated shelf life study was the rational of this experiment.

4.2.13 Statistical analysis

Main effects and interaction effects of treatments [type of starch used to replace the oil (teff and maize), oil replacement level (0%, 50%, 80%, and 100%), and modification of starch (unmodified and modified with 1.5% stearic acid)] were calculated by Analysis of Variance (ANOVA). Means were then compared using Tukey (HSD) test. A t-test was then performed to compare the mean value at D_0 (30 min after preparation) and D_8 (after 8 days of storage at room temperature). The experiments were repeated three times unless stated otherwise.

4.3 Results

4.3.1 Composition of the starches

The chemical composition of commercial maize starch and laboratory extracted teff starch is given in Table 4.2. The protein content of teff starch (1.28%) was higher than maize starch (0.54%). Crude fat and moisture contents were similar for both commercial maize and teff starches.

Table 4.2: Chemical Composition of maize and teff starch (g/100g dry basis)

	Teff	Maize
Crude protein	**1.28 (±0.02)**	**0.54 (±0.02)**
Crude fat	**0.03 (±0.01)**	**0.011 (±0.002)**
Ash	**0.38 (±0.04)**	**0.13 (±0.01)**
Moisture	**2.65 (±0.2)**	**2.93 (±0.2)**
Total starch	**84.2 (±5.7)**	**89.0 (±2.1)**

Values in parenthesis indicate standard deviation of the mean

4.3.2 Pasting properties of teff and maize starches (long pasting)

In this study, 1.5% stearic acid was used to modify teff and maize starches as it was previously found to be the optimum complexation concentration (concentration which achieve the highest final viscosity) (D'Silva. 2009). Figure 4.1 shows the effect of stearic acid on the pasting properties of teff and maize starches. The addition of stearic acid to teff starch resulted in no first viscosity peak compared to maize starch. The addition of stearic acid to maize starch did not affect the first peak, but reduced the breakdown viscosity (the difference between the first peak and trough viscosities).

During the holding time at 91 ^{o}C for 2 h, a small second peak after 70 min was formed for uncomplexed maize starch (Figure 4.1). For uncomplexed teff starch there was no second peak (viscosity increase) during the 2 h holding time at 91 ^{o}C. At the end of the holding time upon cooling to 50 ^{o}C the viscosity of both teff and maize starches increased. The addition of stearic acid to teff and maize starches resulted in continuous increase in viscosity where the second peak did not form completely in the duration of the heating and hold period of the pasting cycle.

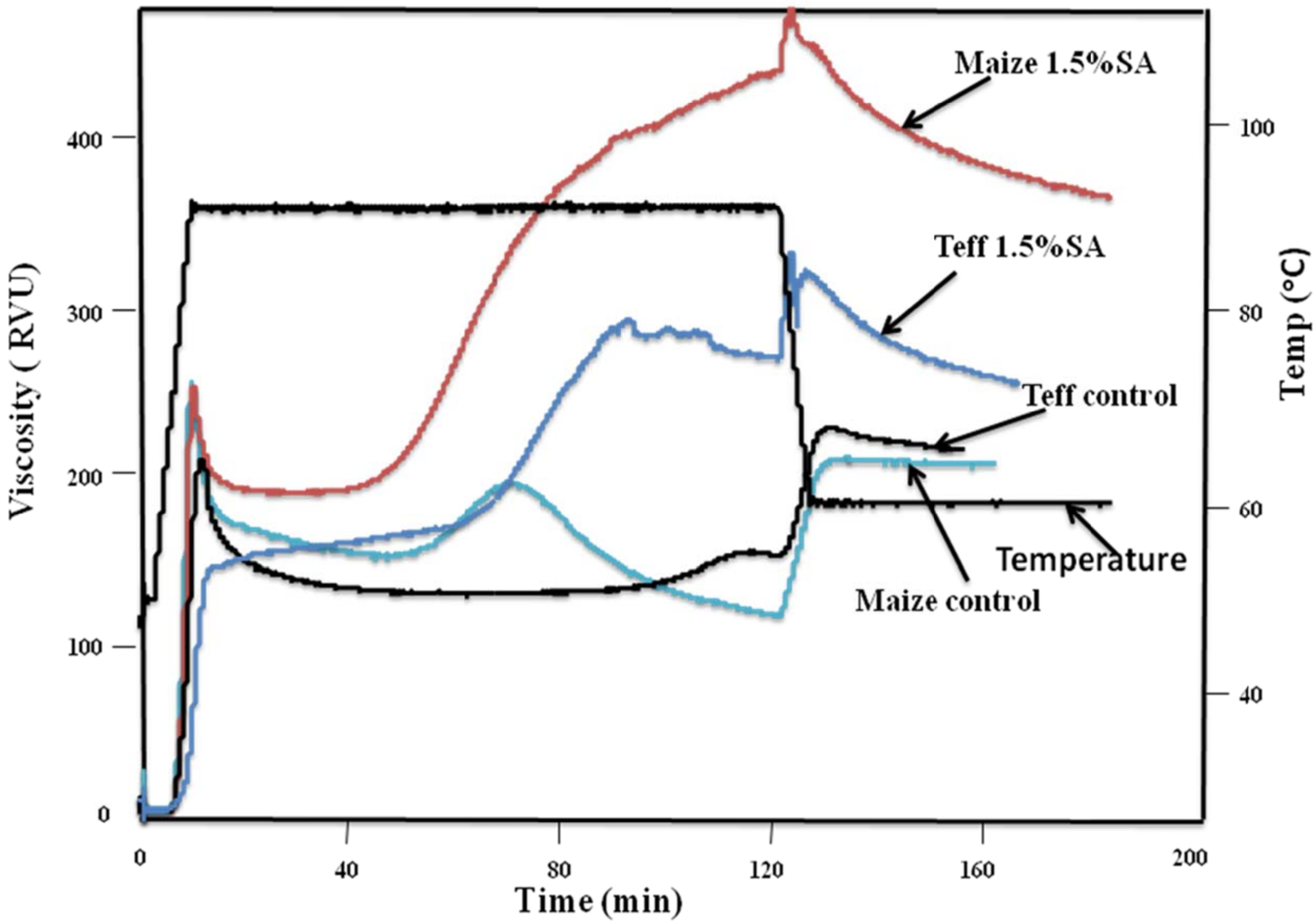


Figure 4.1: Effect of stearic acid on the pasting properties of teff and maize starches.

4.3.3 Flow Properties

In this sub section the results of flow properties of low-calorie mayonnaise type emulsions (LCMTE) measured 30 min and 8 days after preparation will be reported. LCMTE were prepared by replacing 50%, 80% and 100% of the oil with teff and maize starch pastes. The starch pastes used were either unmodified or modified with stearic acid (1.5%). Full fat mayonnaise (reference sample) and a 50% oil reduced product with no starch added (negative control) were used for comparison purposes.

Figures 4.2 and 4.3 show the pouring ability (emptying from a beaker) of each mayonnaise sample 30 min after preparation and after 8 days of storage at room temperature respectively. Eventhough Figures 4.2 and 4.3 are crude ways to present the flow property, this measure can be important as mayonnaise should have some pouring ability for use as a condiment and sauce. The 50% oil reduced product with no starch added poured out of the beaker very fast like water both after 30 min and 8 days of storage (Figures 4.2 B and 4.3B). LCMTE with teff starch modified with stearic acid poured like the full fat mayonnaise at all levels of oil replacement both after 30 min and 8 days of storage (Figures 4.2 D1, D2, D3 and 4.3 D1, D2, D3). LCMTE with modified maize starch poured similar to the full fat mayonnaise only at 50% and 80% oil replacement levels both after 30 min and 8 days of storage (Figures 4.2 F1, F2 and 4.3 F1, F2). However, at 100% oil replacement level LCMTE with modified maize starch flowed slowly compared to the full fat mayonnaise after 30 min and after 8 days storage (Figures 4.2 F3 and 4.3 F3). After 30 min storage LCMTE with unmodified teff starch at 50% and 80% oil replacement level poured out of the beaker (Figure 4.2 C1 and C2), while at 100% oil replacement level did not flow (Figure 4.2 C3). However, after 8 days storage LCMTE with unmodified teff starch did not flow at all levels of oil replacement (Figure 4.3 C1, C2 and C3). LCMTE with unmodified maize starch with oil replacement at 50, 80 and 100% did not flow both after 30 min and 8 days of storage at all levels of oil replacement (Figures 4.2 E1, E2, E3 and 4.3 E1, E2, E3).

The apparent viscosity versus shear rate graphs (Figure 4.4) showed a shear thinning behaviour of all samples studied. The apparent viscosity decreased as the shear rate increased for all samples. The 50% oil reduced product with no starch added had exceptionally low viscosity. This product had also shown phase separation immediately after preparation. Thus, because it did not form a shelf stable emulsion, it was not considered for further discussion.

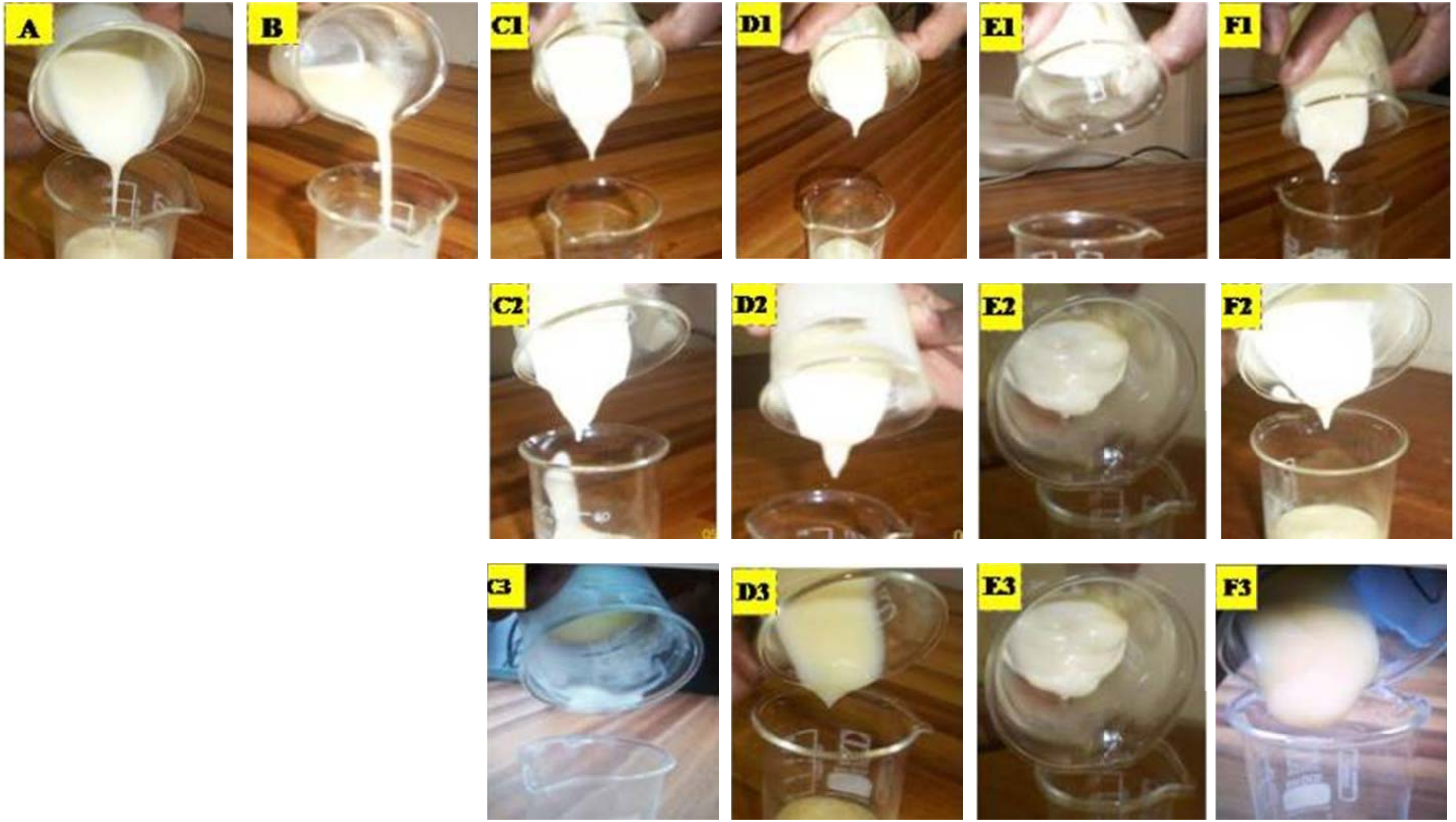


Figure 4.2: Pictures showing pouring ability from a beaker of each mayonnaise sample30 minutes after their preparation. A-full fat mayonnaise, B-50% oil reduced product with no starch added, C1,C2,C3- LCMTEs with unmodified teff starch at 50%, 80%, and 100% oil replacement respectively, D1,D2,D3- LCMTEs with teff starch modified with 1.5% stearic acid at 50%, 80%, and 100% oil replacement respectively, E1,E2,E3- LCMTEs with unmodified maize starch at 50%, 80%, and 100% oil replacement respectively, F1,F2,F3- LCMTEs with maize starch modified with 1.5% stearic acid at 50%, 80%, and 100% oil replacement respectively

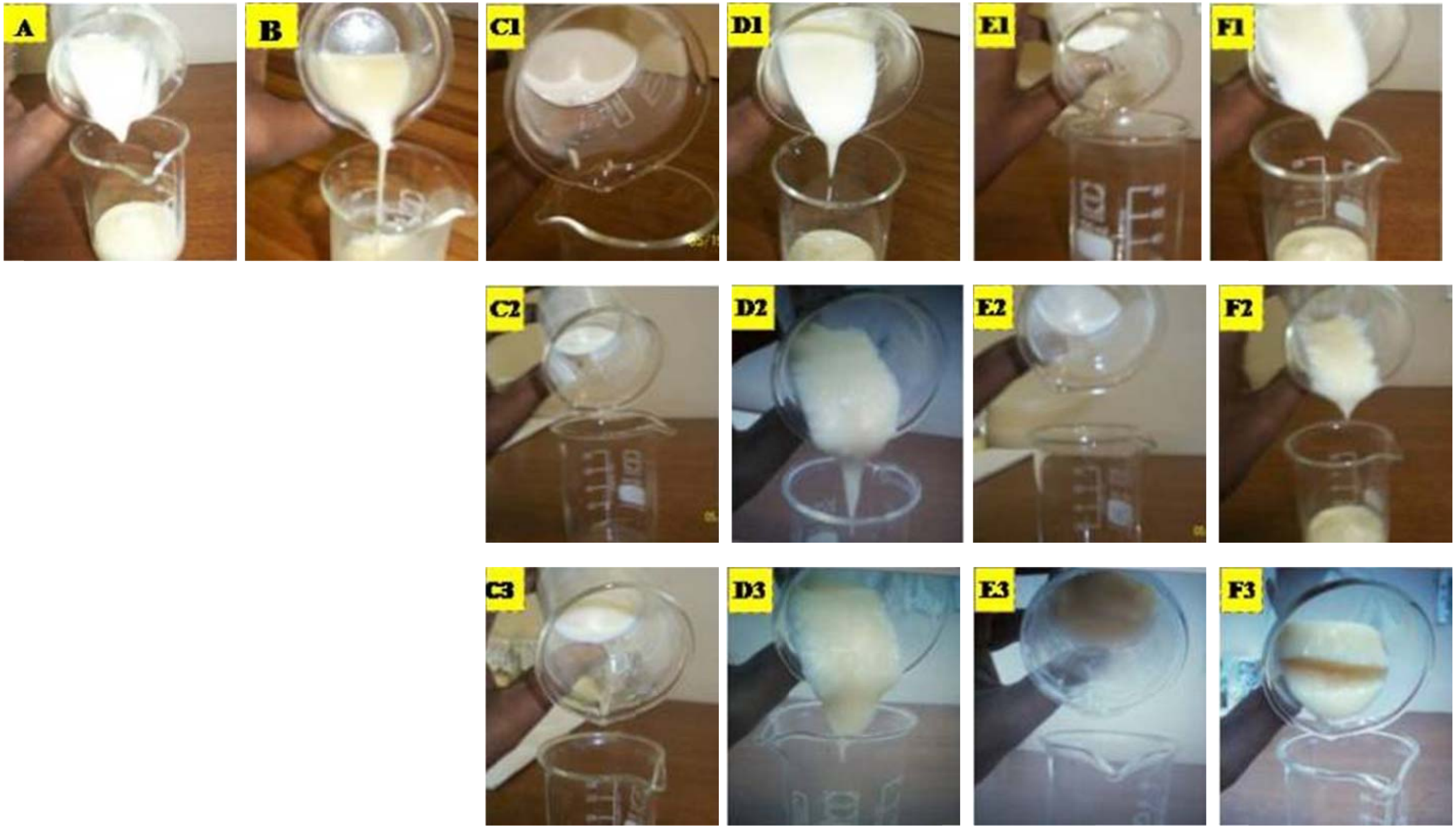


Figure 4.3: Pictures showing pouring ability from a beaker of each mayonnaise sample after 8 days. A-full fat mayonnaise, B- 50% oil reduced product with no starch added, C1,C2,C3- LCMTEs with unmodified teff starch at 50%, 80%, and 100% oil replacement respectively, D1,D2,D3- LCMTEs with teff starch modified with 1.5% stearic acid at 50%, 80%, and 100% oil replacement respectively, E1,E2,E3- LCMTEs with unmodified maize starch at 50%, 80%, and 100% oil replacement respectively, F1,F2,F3- LCMTEs with maize starch modified with 1.5% stearic acid at 50%, 80%, and 100% oil replacement respectively.

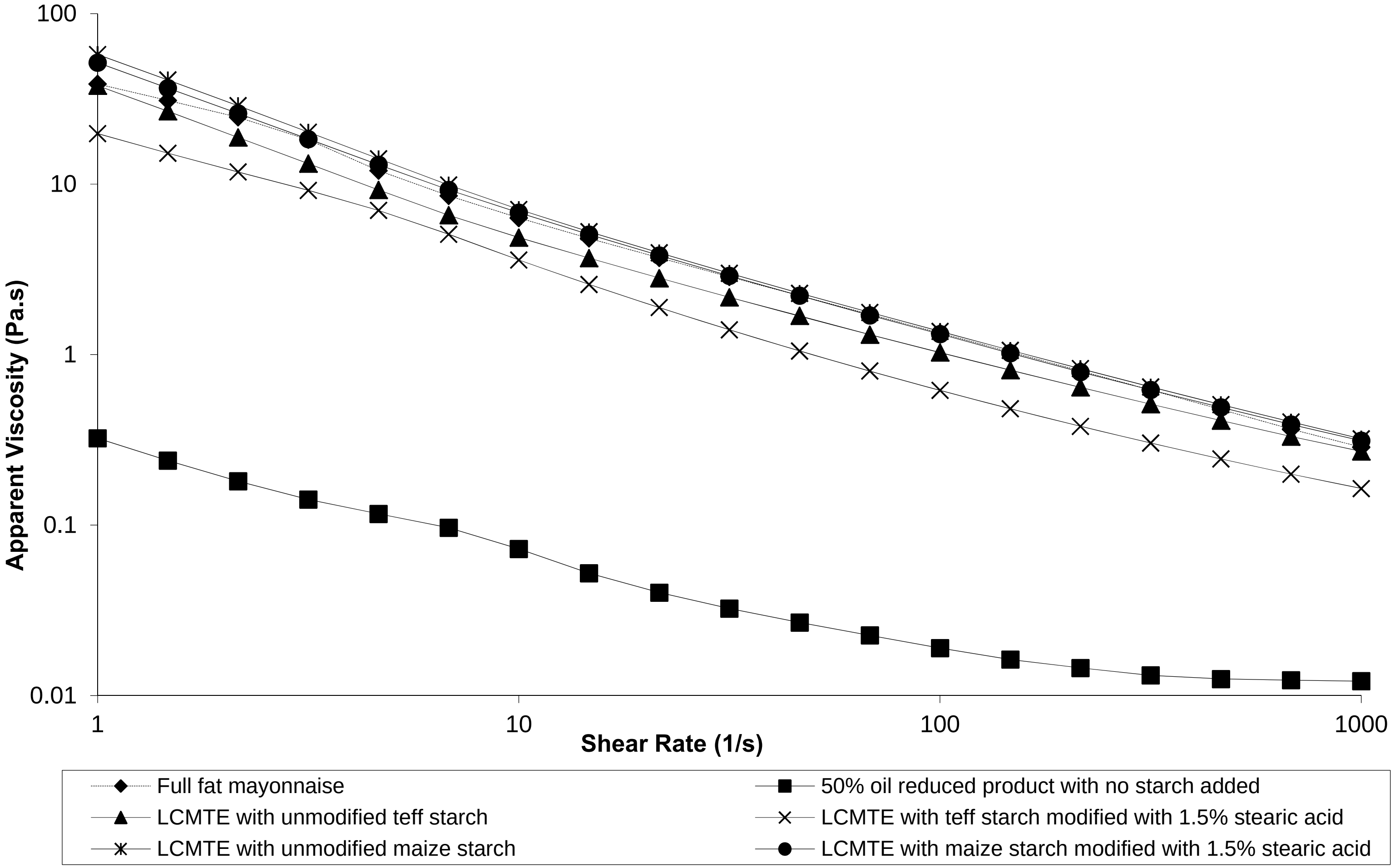


Figure 4.4A: Effect of oil replacement with teff and maize starch pastes on the viscous properties of low calorie mayonnaise type emulsions (LCMTE) at 50% oil replacement level at different shear rates.

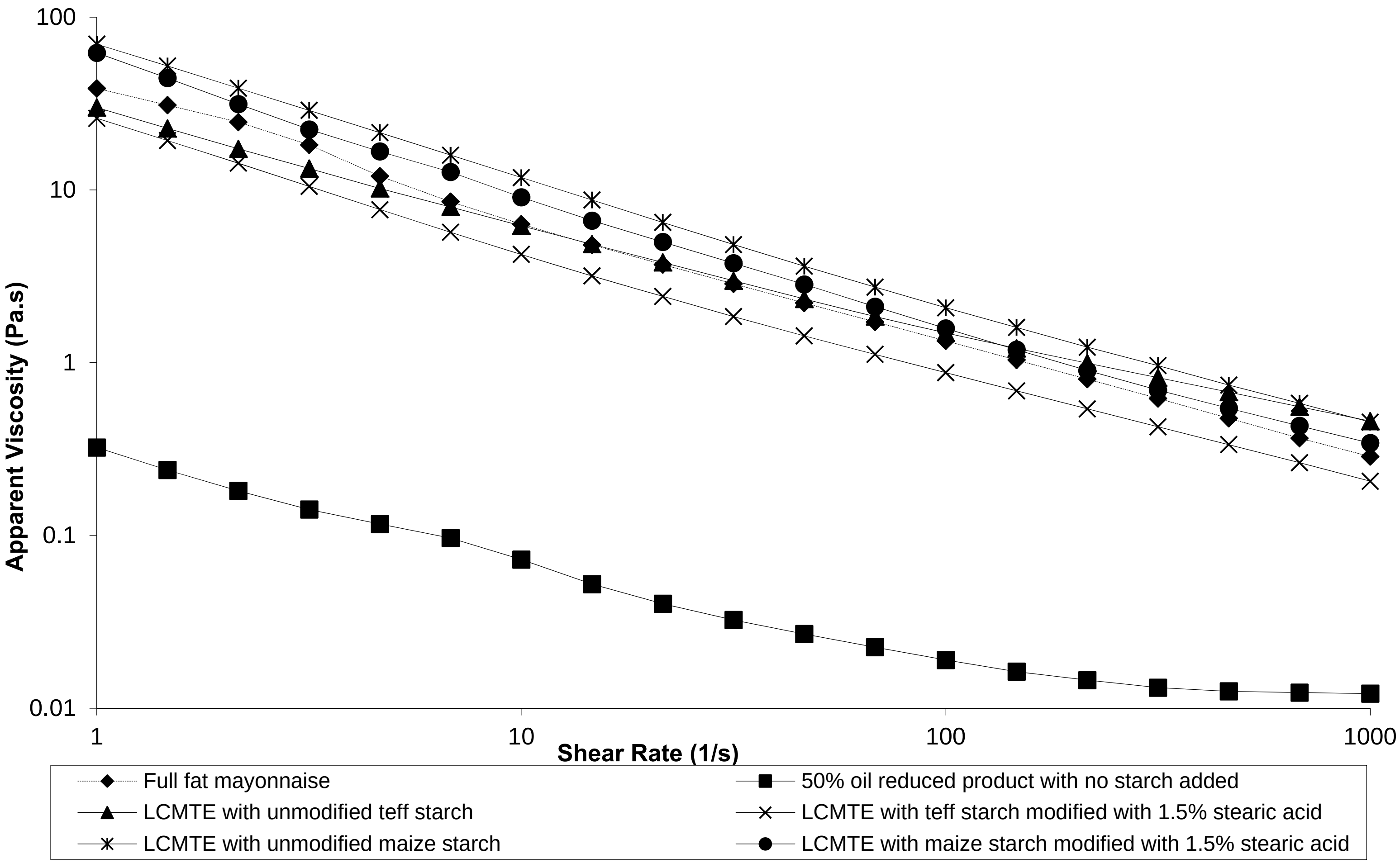


Figure 4.4B: Effect of oil replacement with teff and maize starch pastes on the viscous properties of low calorie mayonnaise type emulsions (LCMTE) at 80% oil replacement level at different shear rates.

**the viscosity vs shear rate curves for full fat mayonnaise and 50% oil reduced product with no starch added was repeated for comparison*.

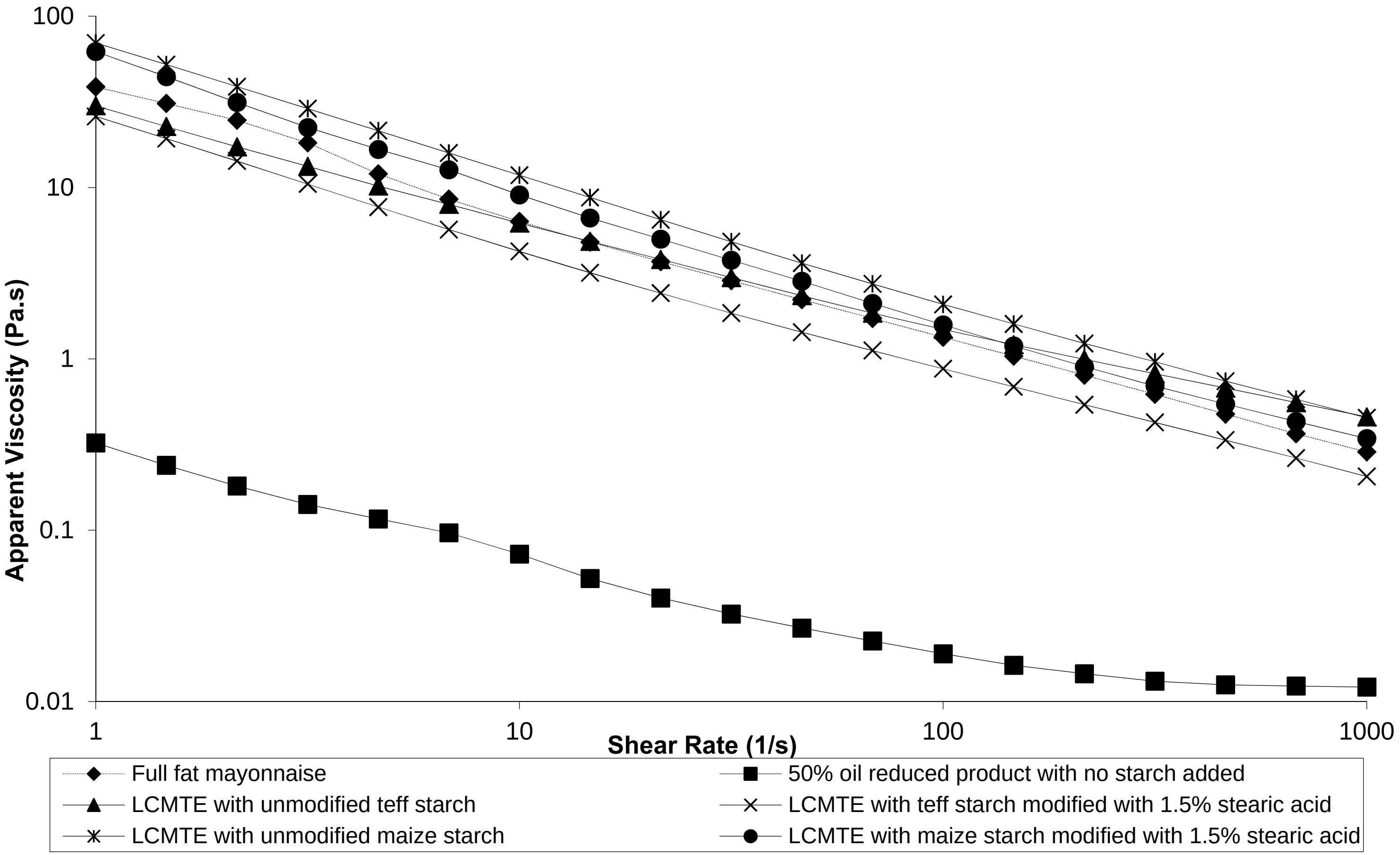


Figure 4.4C: Effect of oil replacement with teff and maize starch on the viscous properties of low calorie mayonnaise type emulsions (LCMTE) at 100% oil replacement level at different shear rates.

****the viscosity vs shear rate curves for full fat mayonnaise and 50% oil reduced product with no starch added was repeated for comparison.***

In the apparent viscosity versus shear rate graphs (Figure 4.4) the shear rate data 0.01 to 1s^{-1} was not used because it had noisy data (ups nad downs).

To characterize the flow properties of mayonnaise both Herschel-Bulkley model (Su et al., 2010, Mun et al., 2009) and Power Law model (Worrasinchai et al., 2006, Maruyama et al., 2007) were used. The Power Law model is a simple model (Branco & Gasparetto, 2003) with a wide technological application. The Herschel-Bulkley model has been largely used in mayonnaise as yield stress is considered (Izidoro et al., 2008). Yield stress is an important quality control parameter for the industrial process, particularly for comparing the overall characteristics of products made on different production lines (Ahmed, 2004). Yield stress is also an important character for mayonnaise when used as salad dressing for it must have a proper ability to stay (be non flowable) on the salad surface (Liu et al., 2007). The Herschel Bulkley and Power Law models may be represented by the following equations:

$\sigma = \sigma_0 + K(\dot{\gamma})^n$ (Herschel-Bulkley model),

$\sigma = K(\dot{\gamma})^n$ (Power Law model)

Where σ_0 is the yield stress; σ is the shear stress (Pa); $\dot{\gamma}$ is the shear rate (1/s); K is the consistency index (Pa.s^n) which is an indication of the viscosity, and n is the flow behavior index where n=1 for Newtonian fluid, n<1 for shear thinning and n>1 for shear thickening materials.

The experimental data (from Figure 4.4) were fitted to Herschel-Bulkley and Power Law models. The flow properties as determined by the two models are summarized in Tables 4.3 to 4.8.

The experimental data showed a good fit to both models as the determination Coefficients (R^2) for both models were higher than 0.90 (0.94 – 0.99 for Herschel-Bulkley and 0.95 – 0.99 for Power Law). However, after 8 days storage LCMTE with unmodified teff starch at 100% oil replacement and LCMTE with modified maize starch with stearic acid at 100% oil replacement did not seem to follow the Herschel-Bulkley model, having exceptionally high K-value (2904 Pa.s^n and 2447 Pa.s^n respectively) and very low n-value (0.01 and 0.07 respectively). Nevertheless, from an overall perspective, the experimental data showed a good fit to both models indicating that the models may be used to discuss the flow properties.

When level of oil replacement increased from 50% to 80% the yield stress decreased and then increased when the oil replacement was 100% for all the LCMTE except for LCMTE with modified teff starch (Table 4.3). Starches modified with stearic acid had lower yield stress ($P<0.05$) compared to unmodified starches (main effect starch modification). LCMTE with teff starches had lower yield stress than LCMTE with maize starch (main effect type of starch). After 8 days storage, the yield stress of most LCMTE increased except for LCMTE with modified teff starch at 80% and 100% oil replacement and LCMTE with modified maize starch at 50% oil replacement.

The Herschel-Bulkley's consistency coefficient (K) value increased with increase in the level of oil replacement for all the samples (Table 4.4). The use of modified starches with stearic acid seemed to decrease the Herschel-Bulkley's consistency coefficient (K) value compared with unmodified starches. LCMTE with teff starches had lower ($P<0.05$) Herschel-Bulkley's consistency coefficient (K) value than LCMTE with maize starches (main effect type of starch). Storage time from D_0 to D_8 seemed to increase the Herschel-Bulkley's consistency coefficient (K) value of most LCMTE except a decrease ($P<0.05$) for LCMTE with modified teff starch at 80% and 100% level of oil replacement. The consistency coefficient (K) value from the Power Law model generally increased with increase in the level of oil replacement on D_0 except for LCMTE with modified teff starch (Table 4.5). The use of modified starches with stearic acid decreased ($P<0.05$) the Power Law model consistency coefficient (K) value compared to unmodified starches (main effect starch modification). LCMTE with teff starches had lower ($P<0.05$) consistency coefficient (K) value from the Power Law model than LCMTE with maize starches (main effect type of starch). Storage time from D_0 to D_8 seemed to increase the consistency coefficient (K) value from Power Law model of most LCMTE except for LCMTE with modified teff starch at 80% and 100% level of oil replacement where a significant decrease ($P<0.05$) was observed.

Table 4.3: Yield stress values (Pa) of mayonnaise samples measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

Storage time									
Day 0					Day 8				
Full Fat mayonnaise		28.09 (1.23)			Full fat mayonnaise		27.7 (2.1)		
Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**	Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**
Teff	50	28.91 (2.9) A	16.56 (0.8) A	17.68 (2.15) B	Teff	50	55.3 (4.5) A*	21.1 (2.1) A	22.08 (4.29) B
	80	18.99 (2.2) A	7.72 (1.2) B			80	21.6 (3.1) B	4.0 (0.2) B*	
	100	26.84 (2.4) A	7.03 (1.2) B			100	27.1 (0.8) B	3.4 (0.2) B*	
Maize	50	46.78 (1.8) A	44.46 (3.0) A	36.39 (2.55) A	Maize	50	49.1 (2.4) B	34.6 (2.3) B	46.32 (3.98) A*
	80	30.64 (1.2) B	19.44 (1.2) C			80	50.3 (2.3) B*	23.7 (0.4) C*	
	100	46.38 (1.5) A	30.60 (0.4) B			100	76.3 (1.1) A*	44.0 (1.8) A*	

		Teff	Maize			Teff	Maize
Main effect modification	Unmodified	24.91 (1.97) A	41.27 (2.77) A	**Main effect Modification**	Unmodified	34.68 (5.46) A	58.54 (4.55) A *
	Modified with stearic acid	10.44 (1.63) B	31.50 (3.74) A		Modified with stearic acid	9.49 (2.96) B	34.10 (3.05) B

- ***Values in parenthesis indicate standard error of the mean***
- ***Means followed by the same letter/s within a block are not significantly different (($P < 0.05$), Tukey (HSD) test.***
- ***Yield stress values followed with * are significantly different from the corresponding values measured at D_0***

Table 4.4: Herschel-Bulkley's consistency coefficient (K) values (Pa.s^n) of mayonnaise samples measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

Storage time										
Day 0						**Day 8**				
Full Fat Mayonnaise		15.63 (0.3)				Full Fat Mayonnaise		14.4 (0.5)		
Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**		Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**
Teff	50	6.08 (0.7) C	6.62 (0.2) B	10.72 (1.02) B		Teff	50	11.4 (1.2) B*	7.3 (0.3) A	21.23 (6.54) B
	80	12.72 (0.8) B	8.74 (0.2) B				80	74.6 (2.1) A*	6.5 (0.4) A*	
	100	17.71 (1.2) A	12.46 (1.0) A				100	-	6.4 (0.1) A*	
Maize	50	9.24 (0.9) B	8.05 (0.6) B	21.64 (3.34) A		Maize	50	16.6 (1.8) B*	10.4 (0.6) B	124.88 (27.83) A*
	80	34.45 (1.3) A	17.51 (0.9) A				80	245 (0.3) A*	82.0 (0.6) A*	
	100	44.14 (6.1) A	16.43 (0.1) A				100	270 (37.1) A*	-	
Main effect modification			Teff	Maize		**Main effect modification**			Teff	Maize
	Unmodified		12.17 (1.75) A	29.28 (5.51) A			Unmodified		42.98 (11.57) A*	177.32 (41.72) A*
	Modified with stearic acid		9.27 (0.90) A	14.00 (1.53) B			Modified with stearic acid		6.73 (0.20) B*	46.22 (13.06) B*

- ***Values in parenthesis indicate standard error of the mean***
- ***Means followed by the same letter/s within a block are not significantly different ((P < 0.05), Tukey (HSD) test.***
- ***Herschel-Bulkley consistency coefficient (K) values followed with * are significantly different from the corresponding values measured at D_0***
- ***'-' Unrealistic Herschel-Bulkley consistency coefficient (K) values.***

Table 4.5: Consistency coefficient (K) values (Pa.s^n) of mayonnaise samples from the *Power Law Model* measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

<table>
<tr><th colspan="11">Storage time</th></tr>
<tr><th colspan="5">Day 0</th><th></th><th colspan="5">Day 8</th></tr>
<tr><td colspan="2">Full Fat Mayonnaise</td><td colspan="3">36.20 (1.9)</td><td></td><td colspan="2">Full Fat Mayonnaise</td><td colspan="3">36.1 (2.2)</td></tr>
<tr><td>Type of starch</td><td>Level of oil replacement</td><td>Unmodified starch</td><td>Modified starch with stearic acid</td><td>Main effect types of starch</td><td></td><td>Type of starch</td><td>Level of oil replacement</td><td>Unmodified starch</td><td>Modified starch with stearic acid</td><td>Main effect types of starch</td></tr>
<tr><td>Teff</td><td>50</td><td>27.68 (1.36) B</td><td>19.84 (0.84) A</td><td></td><td></td><td>Teff</td><td>50</td><td>55.7 (6.3) B*</td><td>24.5 (1.7) A</td><td></td></tr>
<tr><td></td><td>80</td><td>27.44 (1.83) B</td><td>21.35 (1.99) A</td><td>26.76 (2.38) B</td><td></td><td></td><td>80</td><td>76.7 (0.4) B*</td><td>8.9 (0.5) B*</td><td>48.76 (9.84) B*</td></tr>
<tr><td></td><td>100</td><td>46.14 (3.59) A</td><td>18.08 (0.86) A</td><td></td><td></td><td></td><td>100</td><td>118.7 (10.4) A*</td><td>8.1 (0.8) B*</td><td></td></tr>
<tr><td colspan="4"></td><td></td><td></td><td colspan="4"></td><td></td></tr>
<tr><td>Maize</td><td>50</td><td>45.80 (1.27) B</td><td>42.64 (1.72) A</td><td></td><td></td><td>Maize</td><td>50</td><td>57.3 (0.7) C*</td><td>37.3 (1.5) C</td><td></td></tr>
<tr><td></td><td>80</td><td>71.73 (3.55) A</td><td>44.68 (5.54) A</td><td>54.54 (3.22) A</td><td></td><td></td><td>80</td><td>113.0 (3.5) B*</td><td>89.6 (3.0) B*</td><td>97.37 (10.06) A*</td></tr>
<tr><td></td><td>100</td><td>72.32 (1.59) A</td><td>50.05 (1.67) A</td><td></td><td></td><td></td><td>100</td><td>157.6 (7.2) A*</td><td>129.3 (0.5) A*</td><td></td></tr>
<tr><td colspan="5"></td><td></td><td colspan="5"></td></tr>
<tr><td rowspan="3">Main effect modification</td><td colspan="2"></td><td>Teff</td><td>Maize</td><td></td><td rowspan="3">Main effect modification</td><td colspan="2"></td><td>Teff</td><td>Maize</td></tr>
<tr><td colspan="2">Unmodified</td><td>33.75 (3.33) A</td><td>63.29 (4.53) A</td><td></td><td colspan="2">Unmodified</td><td>83.70 (9.90) A*</td><td>109.31 (14.69) A*</td></tr>
<tr><td colspan="2">Modified with stearic acid</td><td>19.76 (0.82) B</td><td>45.79 (2.06) B</td><td></td><td colspan="2">Modified with stearic acid</td><td>13.82 (2.72) B</td><td>85.42 (13.36) A*</td></tr>
</table>

- ***Values in parenthesis indicate standard error of the mean***
- ***Means followed by the same letter/s within a block are not significantly different ((P < 0.05), Tukey (HSD) test.***
- ***K - values followed with * are significantly different from the corresponding values measured at D_0***

All samples studied had a value of $n<1$ from both the Herschel-Bulkley and Power Law models (Table 4.6 and 4.7) indicating all samples were shear thinning. The apparent viscosity versus shear rate curves also showed shear thinning behaviour (Figure 4.4). Generally the n-values (from Herschel-Bulkley) were not affected significantly by level of oil replacement, type of starch (main effect type of starch) and modification with stearic acid (main effect starch modification) except a significant decrease ($P<0.05$) for modified teff starch with stearic acid. Storage time from D_0 to D_8 seemed to decrease the n-value (from Herschel-Bulkley) of most LCMTE except LCMTE with modified teff starches.

The Power Law flow behaviour index (n) values were not affected by modification with stearic acid (Table 4.7 main effect starch modification) and level of oil replacement except LCMTE with unmodified teff starch. However, LCMTE with maize starches had lower ($P<0.05$) n-value (from Power Law) than LCMTE with teff starches (main effect type of starch). Storage time from D_0 to D_8 generally decreased the n-value (from Power Law) of most LCMTE except an increase for modified teff starch with stearic acid at 80% and 100% oil replacement level.

All the mayonnaise samples studied were found to have a thixotropic behaviour over the whole range of shear rate studied ($0.001 - 1000s^{-1}$) (Figure 4.5), where segments of the down curves reached values of shear stress lower than those of the up curves at the same values of shear rate. The magnitude of hysteresis loop area corresponds to an ability to rebuild the damaged structure after the removal of shear forces. A lower hysteresis loop area of mayonnaise corresponds with its ability to rebuild the damaged structure faster after removal of the shear force and vice versa (Abu-Jdayil, 2003). The hysteresis loop area increased (became less stable) with increase in level of oil replacement for LCMTE with modified and unmodified starches generally, except for LCMTE with maize starches (modified and unmodified) at 100% oil replacement (Table 4.8). Addition of stearic acid decreased the hysteresis loop area (become more stable) ($P<0.05$) only for teff starch (main effect starch modification). Type of starch did not affect the hysteresis loop area significantly (main effect type of starch). Storage for 8 days increased the hysteresis loop area (became less stable) of most LCMTE except no significant change for modified teff and maize starches with stearic acid at 80% replacement level, and a decrease (became more stable) ($P<0.05$) for modified teff starch with stearic acid at 100% oil replacement level.

Table 4.6: Flow behaviour index (n) values of mayonnaise samples from Herschel-Bulkley model, measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

Storage time										
Day 0						**Day 8**				
Full Fat Mayonnaise		0.43 (0.02)				Full Fat Mayonnaise		0.44 (0.01)		
Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**		Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**
Teff	50	0.54 (0.02) A	0.44 (0.003) A	0.46 (0.01) A		Teff	50	0.44 (0.03) A	0.41 (0.01) A*	0.39 (0.04) A*
	80	0.51 (0.01) AB	0.42 (0.01) AB				80	0.23 (0.01) B*	0.44 (0.02) A	
	100	0.45 (0.01) B	0.39 (0.01) B				100	-	0.42 (0.04) A	
Maize	50	0.49 (0.01) A	0.51 (0.01) A	0.41 (0.03) A		Maize	50	0.40 (0.01) A*	0.44 (0.01) A*	0.26 (0.04) B*
	80	0.39 (0.02)A	0.45 (0.07) A				80	0.16 (0.03) B*	0.20 (0.03) B*	
	100	0.23 (0.1) A	0.37 (0.03) A				100	0.10 (0.02) B	-	
Main effect modification			Teff	Maize		**Main effect modification**			Teff	Maize
	Unmodified		0.50 (0.01) A	0.37 (0.05) A			Unmodified		0.34 (0.06) A*	0.22 (0.05) A*
	Modified with stearic acid		0.42 (0.01) B	0.44 (0.03) A			Modified with stearic acid		0.43 (0.01) A	0.32 (0.06) A

- ➢ *Values in parenthesis indicate standard error of the mean*
- ➢ *Means followed by the same letter/s within a block are not significantly different ((P < 0.05), Tukey (HSD) test.*
- ➢ *n - values followed with * are significantly different from the corresponding values measured at D_0*

Table 4.7: Flow behaviour index (n) values of mayonnaise samples from Power Law model, measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

Storage time							
		Day 0			**Day 8**		
Full Fat Mayonnaise		0.28 (0.01)			0.30 (0.01)		
Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**
Teff	50	0.30 (0.01) B	0.28 (0.003) B	0.32 (0.01) A	0.19 (0.01) A*	0.24 (0.01) B*	0.26 (0.02) A*
	80	0.38 (0.01) A	0.31 (0.01) AB		0.20 (0.01) A*	0.39 (0.02) A*	
	100	0.31 (0.01) B	0.33 (0.01) A		0.16 (0.01) A*	0.40 (0.03) A	
Maize	50	0.25 (0.003) A	0.26 (0.003) A	0.25 (0.01) B	0.22 (0.003) A*	0.24 (0.01) A	0.20 (0.01) B*
	80	0.28 (0.02) A	0.24 (0.01) A		0.20 (0.02) A	0.19 (0.01) AB	
	100	0.23 (0.03) A	0.24 (0.01) A		0.18 (0.01) A	0.14 (0.02) B*	

		Day 0		**Day 8**	
		Teff	Maize	Teff	Maize
Main effect modification	Unmodified	0.33 (0.01) A	0.26 (0.01) A	0.18 (0.01) B*	0.20 (0.01) A*
	Modified with stearic acid	0.31 (0.01) A	0.25 (0.01) A	0.34 (0.03) A	0.19 (0.02) A*

- ***Values in parenthesis indicate standard error of the mean***
- ***Means followed by the same letter/s within a block are not significantly different (($P < 0.05$), Tukey (HSD) test.***
- ***n - values followed with * are significantly different from the corresponding values measured at D_0***

Table 4.8: Hysteresis loop areas (Pa/s) of mayonnaise samples, measured 30 minutes after preparation (D_0) and after 8 days of storage (D_8) at room temperature

Storage time									
Day 0					**Day 8**				
Full Fat Mayonnaise		19124 (660)			Full Fat Mayonnaise		22728 (1203)		
Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**	Type of starch	Level of oil replacement	Unmodified starch	Modified starch with stearic acid	**Main effect types of starch**
Teff	50	15963 (1674) C	8651 (503) B		Teff	50	31628 (2443) C*	16077(750) A*	
	80	50674 (3163) B	11094 (825) B	27319 (5048) A		80	65726 (2747) B*	11033 (2762)A	34789 (6439) B
	100	61321 (1226) A	16211 (177) A			100	75233 (406) A*	9039 (371) A*	
Maize	50	16979 (1385) C	19540 (1340) C	35850 (4005) A	Maize	50	34773 (1451) B*	27823 (1535) B*	58396 (6290) A*
	80	63623 (717) A	44601 (537) A			80	92262 (4655) A*	57968 (6327) A	
	100	43759 (1472) B	26595 (1000) B			100	91410 (2217) A*	46141 (2073) A*	
Main effect modification			Teff	Maize	**Main effect modification**			Teff	Maize
	Unmodified		42653 (6934) A	41454 (6785) A		Unmodified		57529 (6705) A*	72815 (9636) A*
	Modified with stearic acid		11985 (1149) B	30245 (3765) A		Modified with stearic acid		12050 (1338) B	43977 (4808) B*

- ➢ ***Values in parenthesis indicate standard error of the mean***
- ➢ ***Means followed by the same letter/s within a block are not significantly different ((P < 0.05), Tukey (HSD) test.***
- ➢ ***Hysteresis loop area values followed with * are significantly different from the corresponding values measured at D_0***

4.3.4 Mayonnaise Microscopy

The optical and confocal laser scanning microscopy (CLSM) images showed similar structures but the CLSM gave clearer images because the possibility of optical sectioning in CLSM eliminates the drawbacks of conventional fluorescence microscopy in observing thick specimens. Full fat mayonnaise after 24 hr of preparation showed closely packed and uniformly distributed oil droplets of about 5-10 µm (Figures 4.6 and 4.10). The 50% oil reduced product with no starch added had large non uniform oil droplets of about 20-40 µm, and larger proportion of aqueous phase compared to full fat mayonnaise. This shows phase separation. When the level of oil replacement increased from 50% to 80% the oil phase decreased because of decrease in oil content and at 100% oil replacement level there were no oil droplets (Figures 4.6, 4.7, 4.10 and 4.11). Compared to full fat mayonnaise LCMTE with 50% and 80% oil replacement level had smaller oil droplets (1-10 µm) (Figures 4.10 C, D and 4.11 C, D, F, G) except LCMTE with modified teff starch (10-20 µm) (Figure 4.10 F and G). In LCMTE with unmodified maize starch at 100% oil replacement level some discrete bodies which could be remnant starch granules/ghost were observed (Figure 4.11 E). LCMTE with modified starches at 50% and 80% oil replacement levels had larger size oil droplets compared to LCMTE with unmodified starches. LCMTE with maize starches (Figure 4.11) seemed to have smaller oil droplets compared to LCMTE with teff starches (Figure 4.10) especially with the modified starches. After storage for 8 days, the oil droplets size increased in full fat mayonnaise, the 50% oil reduced product with no starch added and LCMTE with modified teff starch at 50% and 80% oil replacement levels (Figures 4.8, 4.9, 4.12 and 4.13).

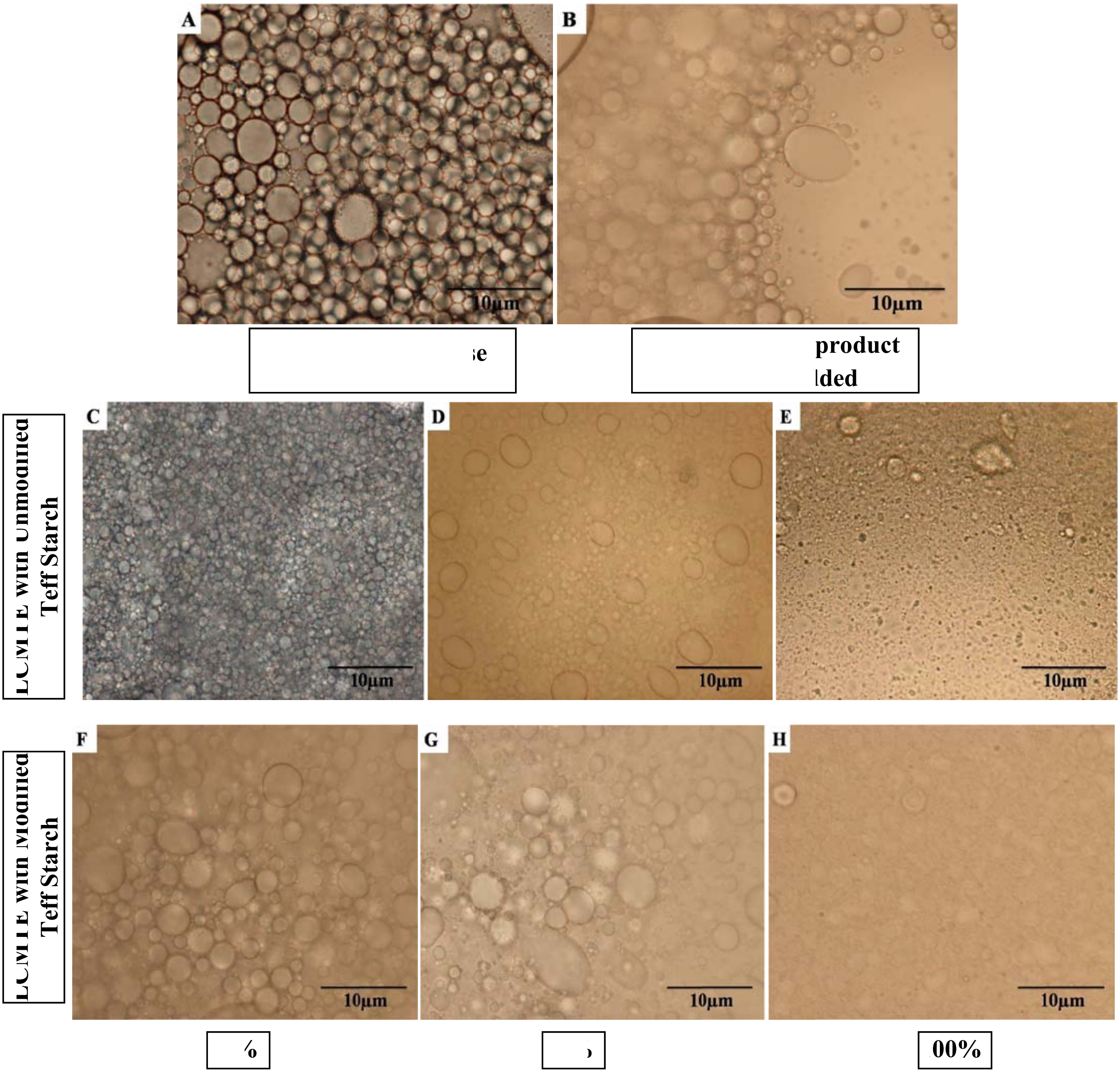


Figure 4.5: Optical microscopy images of (after one days of storaage at room temperature): A - Full fat Mayonnaise, B – 50% reduced oil product with no starch added, C, D, E - LCMTE with unmodified teff starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with teff starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively .Magnifications 40X.

**the droplets represent the oil phase and the remaining empty space represent the aqueous phase.*

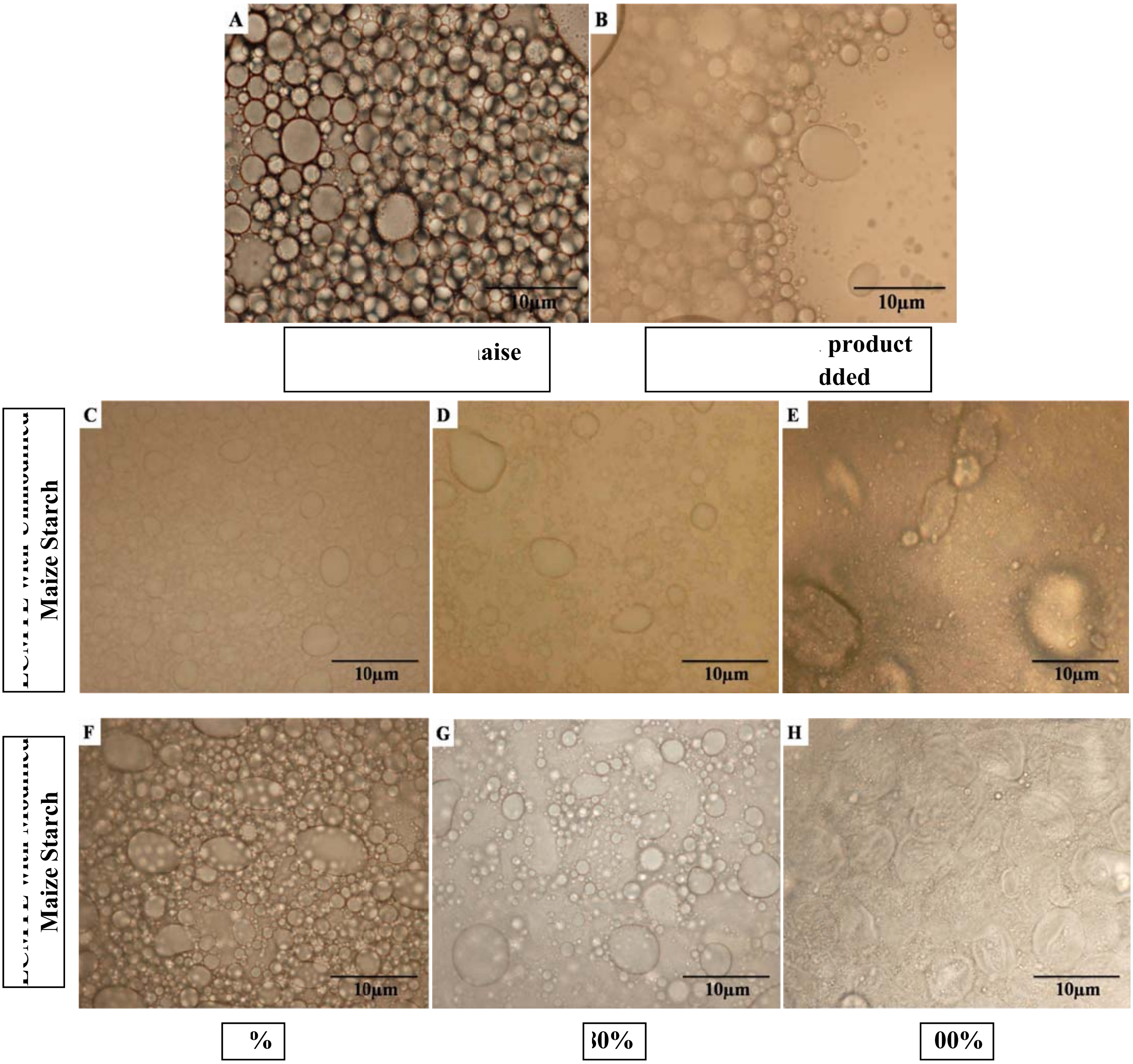


Figure 4.6: Optical microscopy images of (after one days of storaage at room temperature): A - Full fat Mayonnaise, B –50% oil reduced product with no starch added, C, D, E - LCMTE with unmodified maize starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with maize starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively .Magnifications 40X.

**the droplets represent the oil phase and the remaining empty space represent the aqueous phase.*

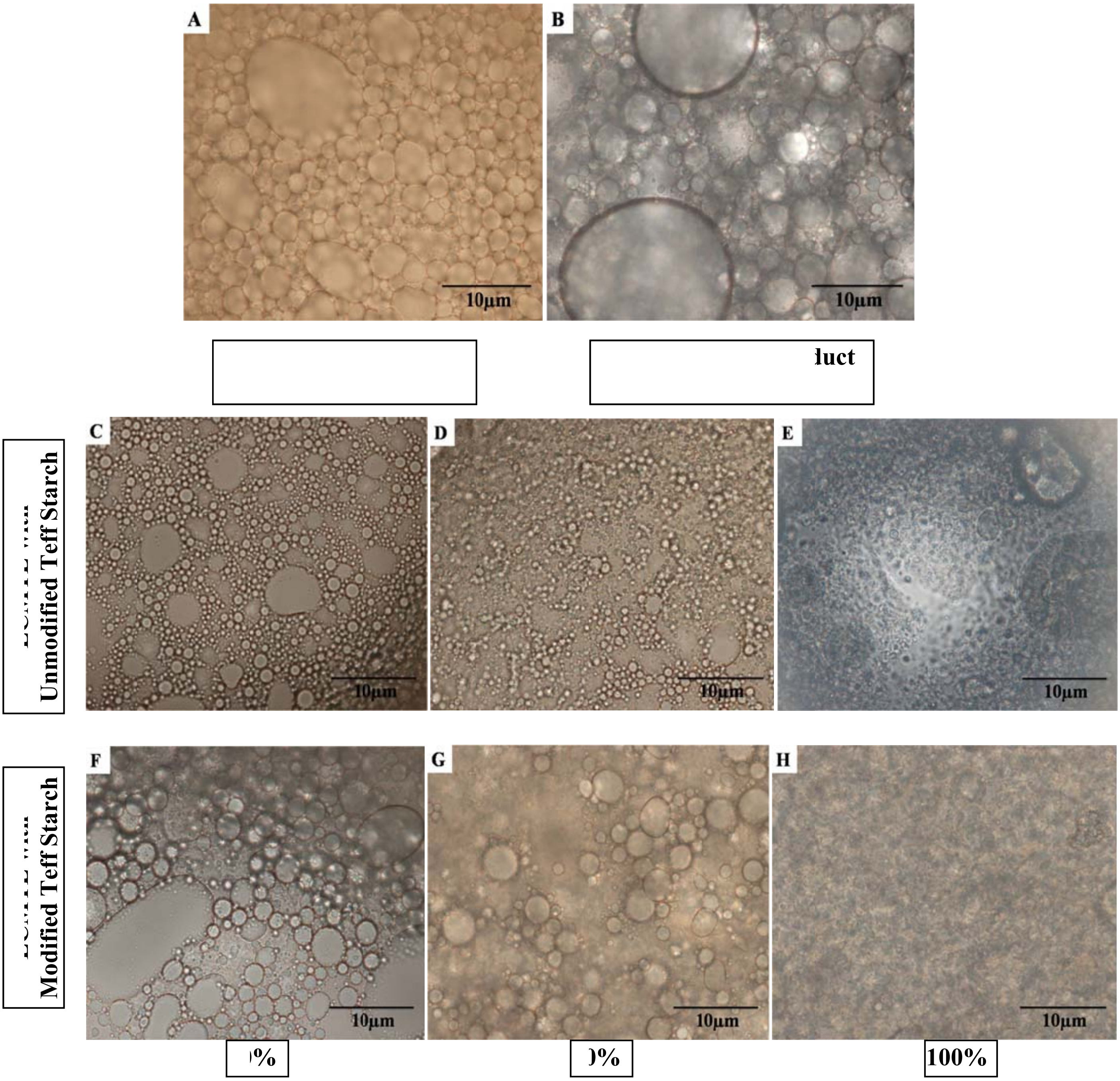


Figure 4.7: Optical microscopy images of (after 8 days of storaage at room temperature): A - Full fat Mayonnaise, B – 50% oil reduced product with no starch added, C, D, E - LCMTE with unmodified teff starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H- LCMTE with teff starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively .Magnifications 40X.

**The droplets represent the oil phase and the remaining empty space represent the aqueous phase.*

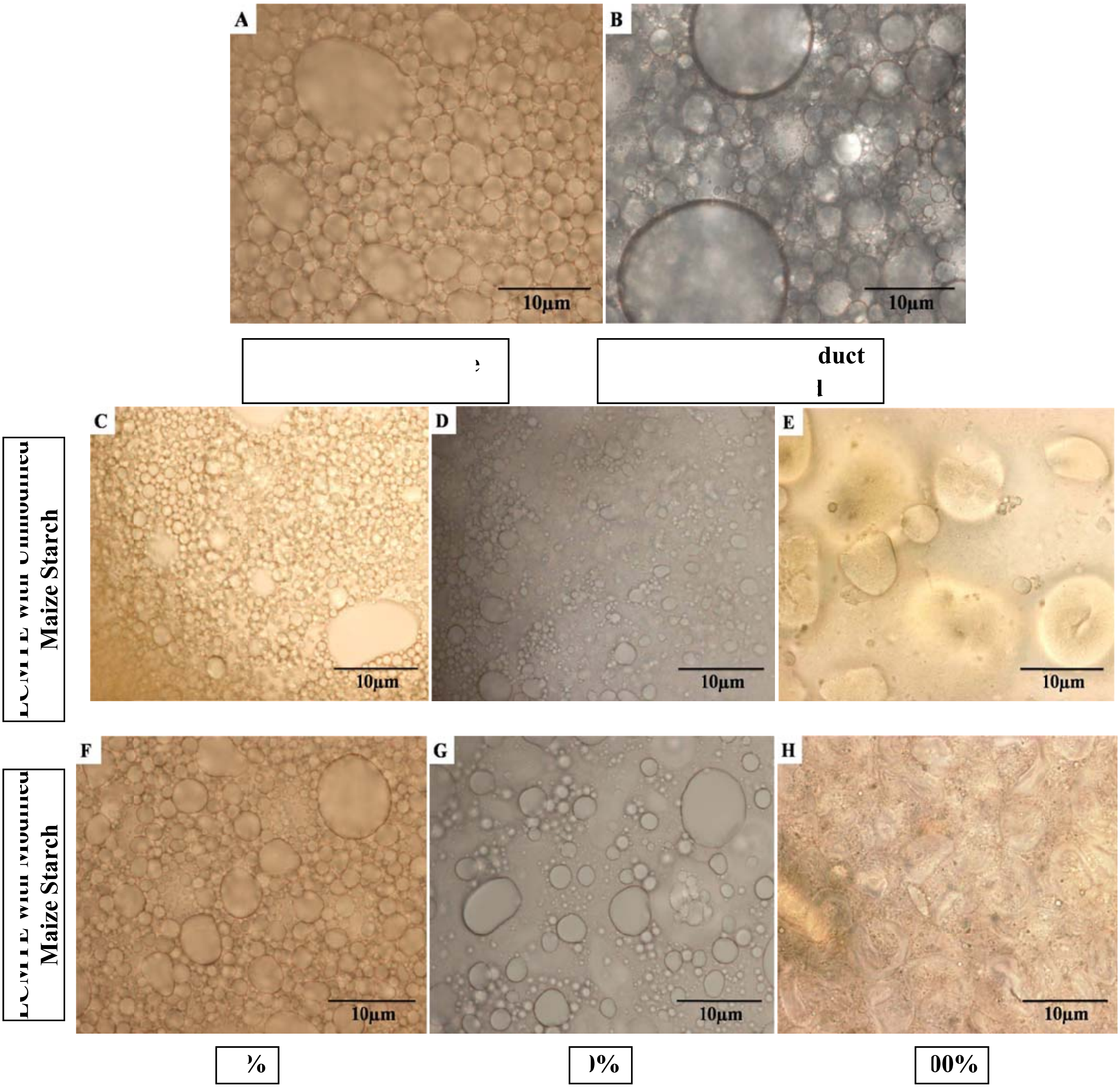


Figure 4.8: Optical microscopy images (after 8 days storaage at room temperature): A - Full fat Mayonnaise, B –50% oil reduced product with no starch added, C, D, E - LCMTE with unmodified maize starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with maize starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively. Magnifications 40X.

**The droplets represent the oil phase and the remaining empty space represent the aqueous phase.*

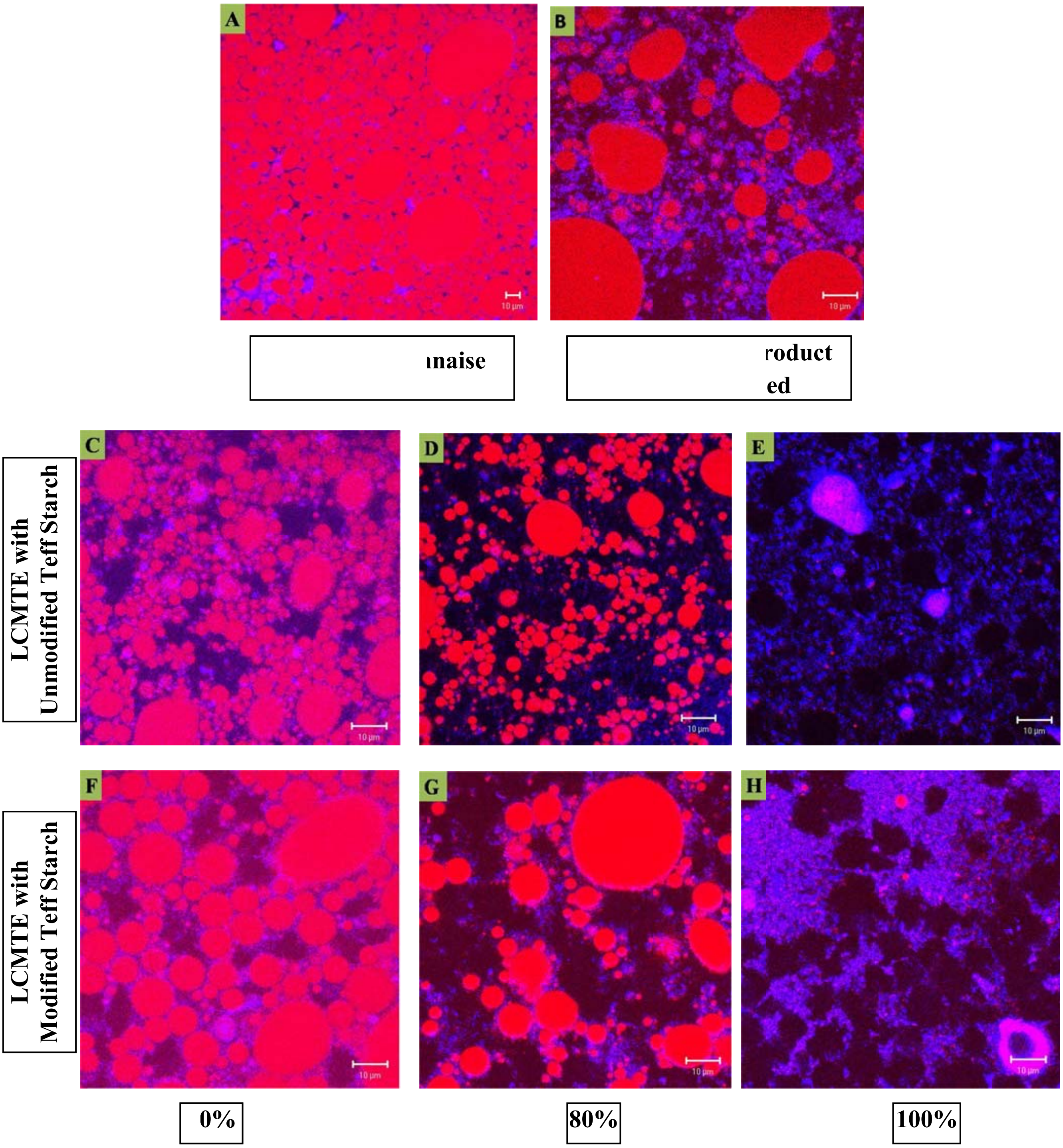


Figure 4.9: CLSM images of (taken after one day storage): A - Full fat Mayonnaise, B – reduced oil product with no starch added, C, D, E - LCMTE with unmodified teff starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with teff starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively.

**The droplets stained red represent the oil phase and the blue space represent the aqueous phase.*

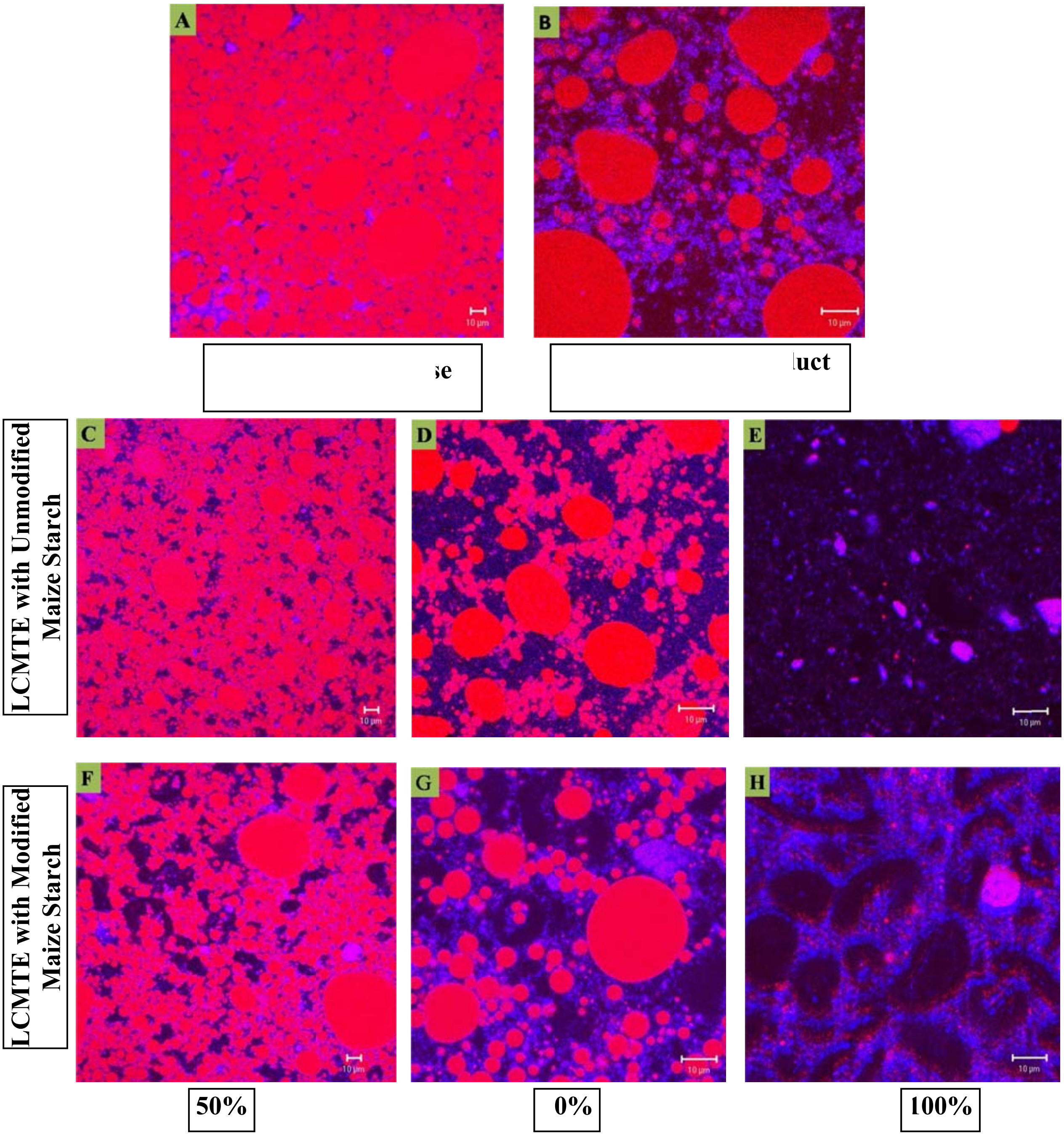


Figure 4.10: CLSM images of (taken after one day storage): A - Full fat Mayonnaise, B – reduced oil product with no starch added, C, D, E - LCMTE with unmodified maize starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with maize starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively.

**The droplets stained red represent the oil phase and the blue space represent the aqueous phase.*

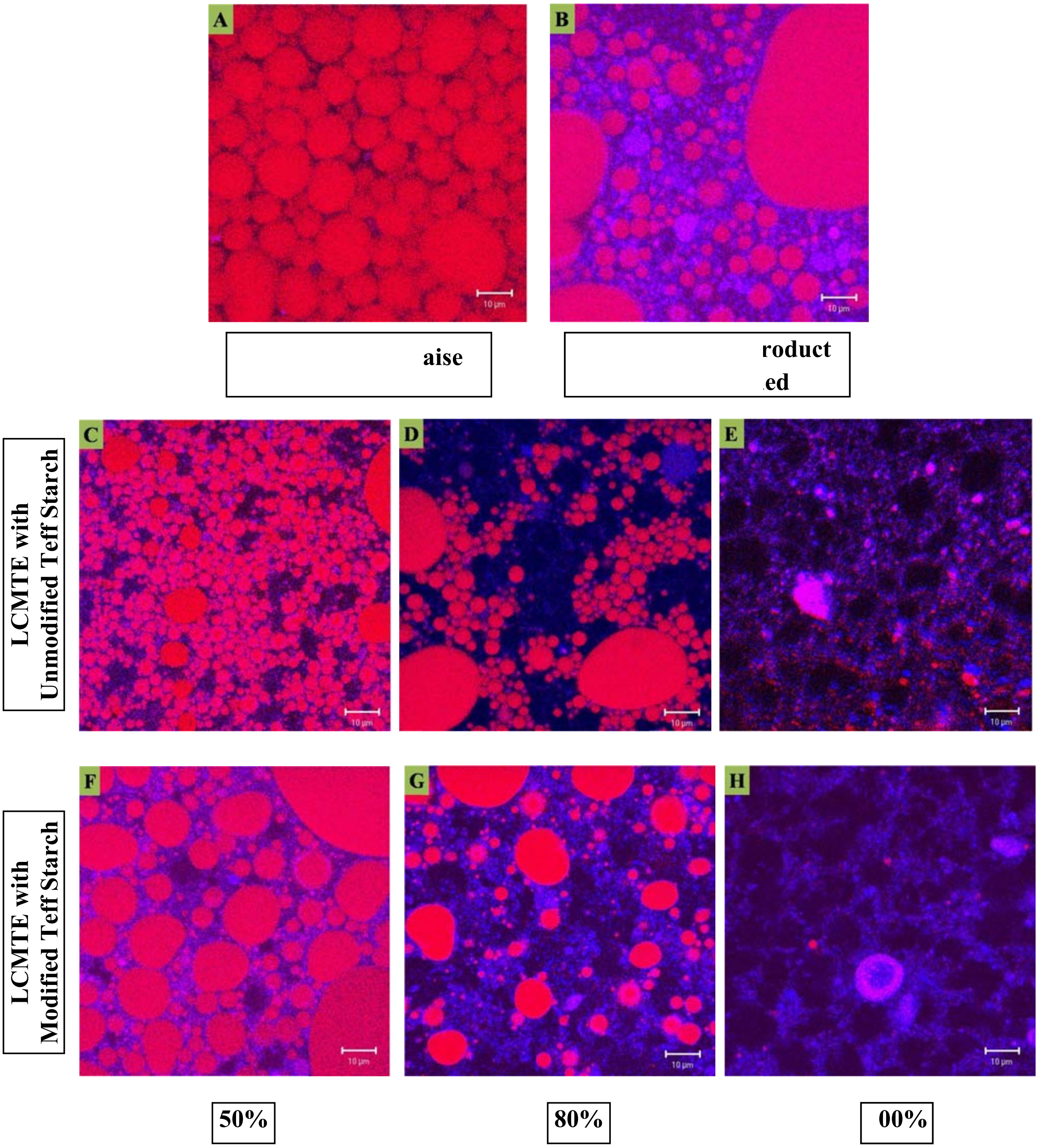


Figure 4.11: CLSM images of (taken after 8 days storage): A - Full fat Mayonnaise, B – reduced oil product with no starch added, C, D, E - LCMTE with unmodified teff starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with teff starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively.

**The droplets stained red represent the oil phase and the blue space represent the aqueous phase.*

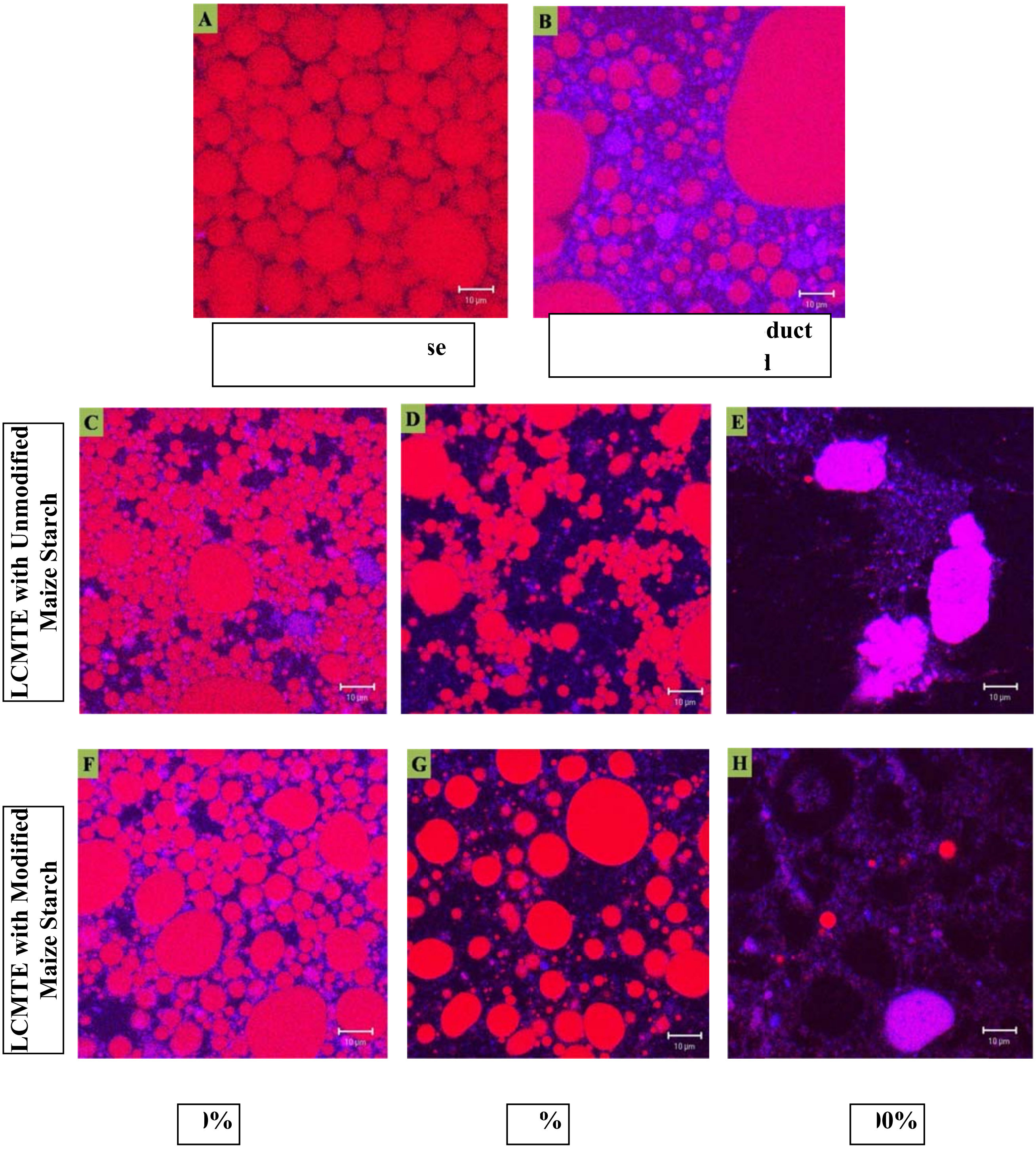


Figure 4.12: CLSM images of (taken after 8 days storage): A - Full fat Mayonnaise, B – reduced oil product with no starch added, C, D, E - LCMTE with unmodified maize starch pastes at 50%, 80% and 100% oil replacement level respectively, F, G, H-LCMTE with maize starch paste modified with 1.5% stearic acid at 50%, 80% and 100% oil replacement level respectively.

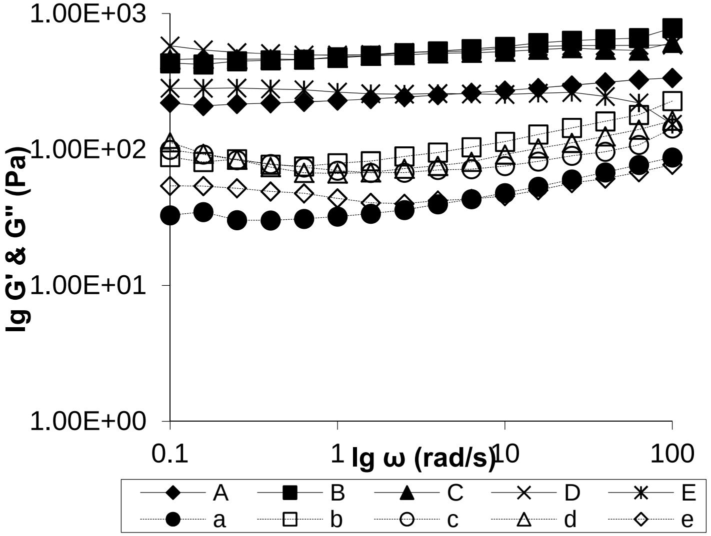
**The droplets stained red represent the oil phase and the blue space represent the aqueous phase*

4.3.5 Viscoelastic Properties

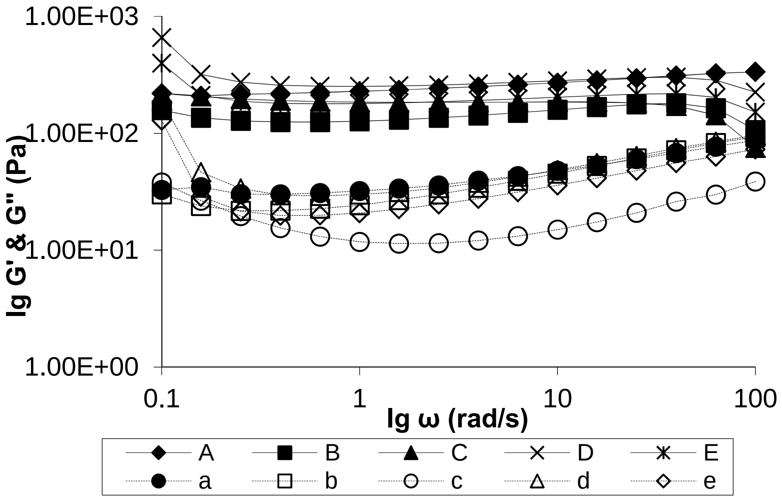
The linear viscoelastic (LVE) range was determined with strain amplitude sweep. Then frequency sweep tests were conducted using strain amplitude of 0.5%, which is within the LVE, to characterize the viscoelastic properties of the full fat mayonnaise and LCMTE containing teff and maize starch pastes. The results were expressed in terms of the elastic modulus (G') and loss modulus (G"). If G' > G", the material exhibits a solid like behaviour; however, if G" > G', the material behaves like a liquid (Mezger, 2006). The results can also be expressed as the ratio of the loss modulus and the storage modulus (G"/G') which is known as “tangent delta” or tanδ. For materials behaving as liquid tanδ > 1, for materials which behave as solid (gel) tanδ < 1 and tanδ = 1 at gel point (Mezger, 2006).

All samples had higher G' than G" throughout the tested frequency range and a tanδ value of less than unity (Figures 4.14 and 4.15). Thus, all the samples behaved like a solid rather than a liquid. With increasing level of oil replacement from 50% to 80% G', G" and tanδ decreased and then when the oil replacement level was increased to 100% G', G" and tanδ increased except LCMTE with modified teff starch, where the G' and G" showed a decrease. Generally LCMTE with modified starches had lower G', G" and tanδ than LCMTE with unmodified starches.

i)



ii)



iii)

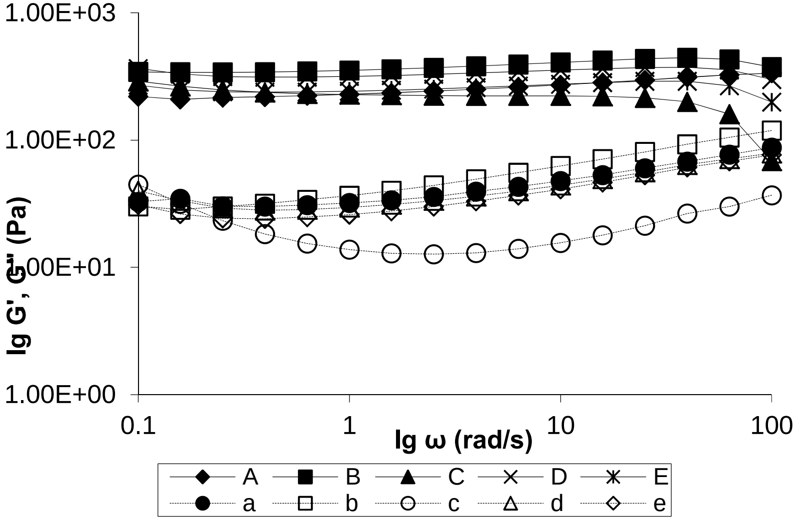
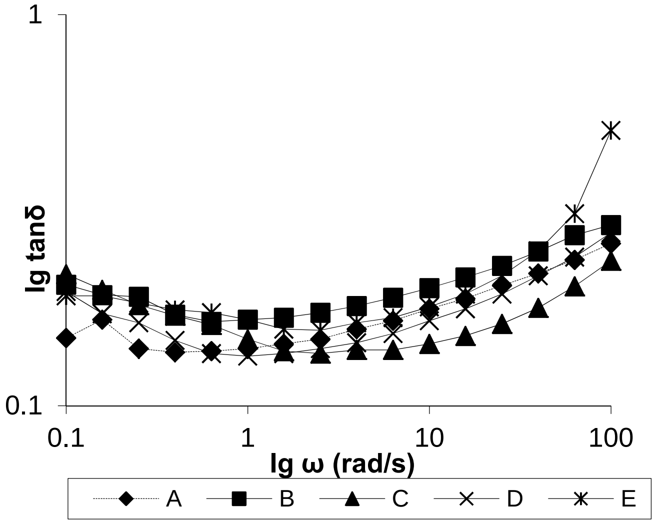


Figure 4.13: Dynamic oscillatory responces (G' and --- G'' ---) of mayonnaise samples, A (a) full fat mayonnaise, B (b) LCMTE with unmodified teff starch, C (c) LCMTE with teff starch modified with 1.5% stearic acid, D (d) LCMTE with unmodified maize starch, and E (e) LCMTE with maize starch modified with 1.5% stearic acid. i) 50% oil replacement level, ii) 80% oil replacement level and iii) 100% oil replacement level.

****For comparison the graph for full fat mayonnaise is repeated at 80% and 100% oil replacement levels.***

i)



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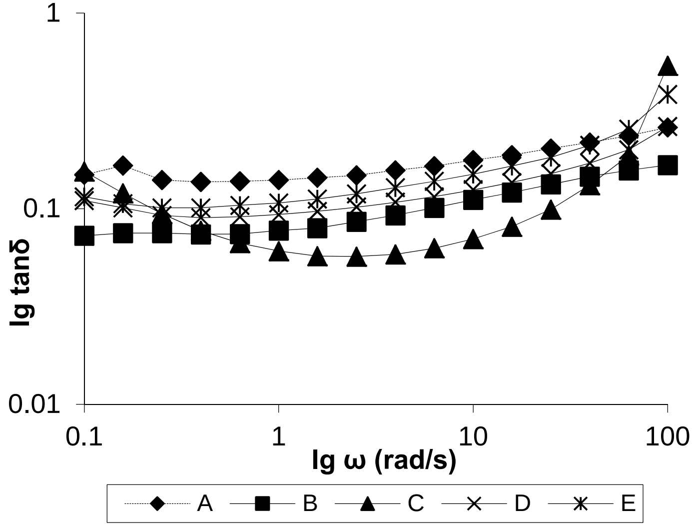

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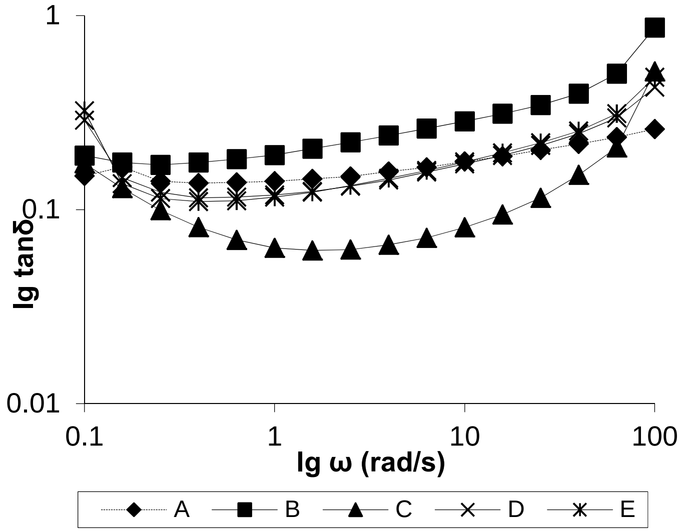


Figure 4.14: Dynamic oscillatory responces (tan δ) of mayonnaise samples, A- full fat mayonnaise, B- LCMTE with unmodified teff starch, C- LCMTE with teff starch modified with 1.5% stearic acid, D- LCMTE with unmodified maize starch, and E- LCMTE with maize starch modified with 1.5% stearic acid. i) 50% oil replacement level, ii) 80% oil replacement level and iii) 100% oil replacement level.

**For comparison the graph for full fat mayonnaise is repeated at 80% and 100% oil replacement levels.*

4.3.6 Freeze-thaw Stability

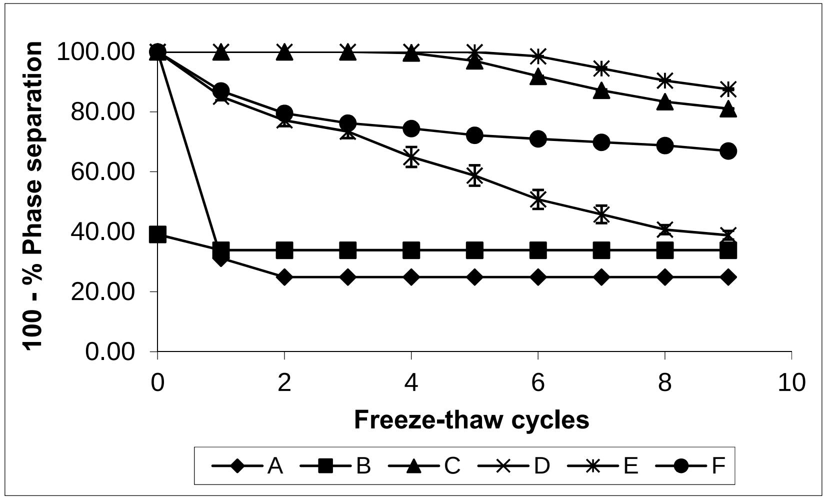
All the samples were subjected to nine consecutive freeze-thaw cycles and the amount of phase separation at each freeze-thaw cycle was measured.

The 50% oil reduced product with no starch added showed phase separation (60% phase separation) at the initial stage, i.e. before any freeze-thaw cycle (Figure 4.16). Full fat mayonnaise showed phase separation at the 1st freeze-thaw cycle (60% phase separation). Generally with increase in oil replacement level from 50% to 80% the phase separation had decreased and then increased when increasing to 100% oil replacement level at each freeze-thaw cycle. LCMTE with modified starches showed more phase separation (start at 1st freeze-thaw cycle with 15% phase separation) than LCMTE with unmodified starches (start at 5th and 6th cycles with 3% and 1.5% phase separation respectively for teff and maize starches) at 50% oil replacement level. At 80% oil replacement level all except LCMTE with modified teff starch showed no phase separation after 10 freeze-thaw cycles. At 100% oil replacement level LCMTE with unmodified starches showed more phase separation than LCMTE with modified starches. LCMTE with maize starches showed less phase separation than LCMTE with teff starch at 50% oil replacement level. At 100% oil replacement level LCMTE with modified teff starch had more phase separation (10% phase separation at 1st cycle) than LCMTE with modified maize starch (3% phase separation at 1st cycle) while LCMTE with unmodified maize starch had more phase separation after the 2nd cycle (11% phase separation at 2nd cycle) than LCMTE with unmodified teff starch (8% phase separation at 2nd cycle).

4.3.7 High Temperature Stability

The 50% oil reduced product with no starch added showed phase separation (50.5% phase separation) after 10 min storage at 50 °C (Figure 4.17). Full fat mayonnaise also showed some phase separation after 10 min storage at 50 °C (9.5% phase separation). LCMTE with modified teff starch showed phase separation after 14 days storage at 50 °C (10.5% phase separation) at 50% oil replacement level and after 1 day storage (8% phase separation) at 100% oil replacement level. LCMTE with modified teff starch at 80% oil replacement level and all the other LCMTE at all levels of oil replacement seemed to have no phase separation.

i)



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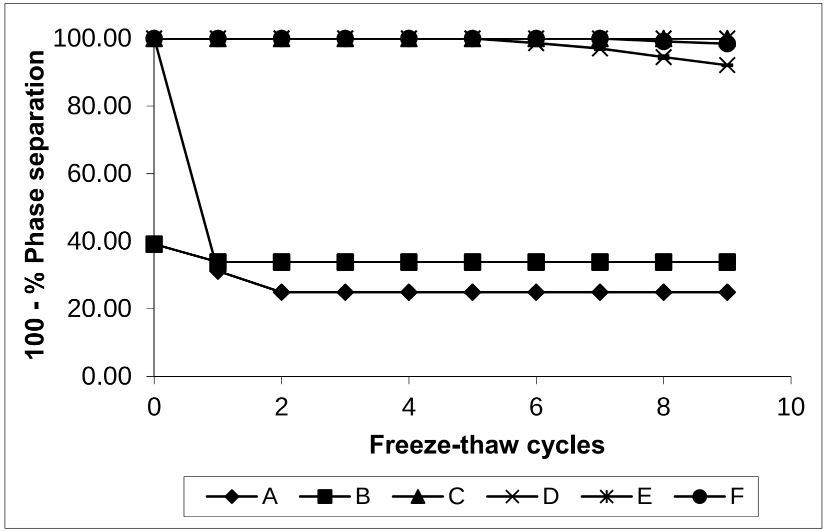


iii)

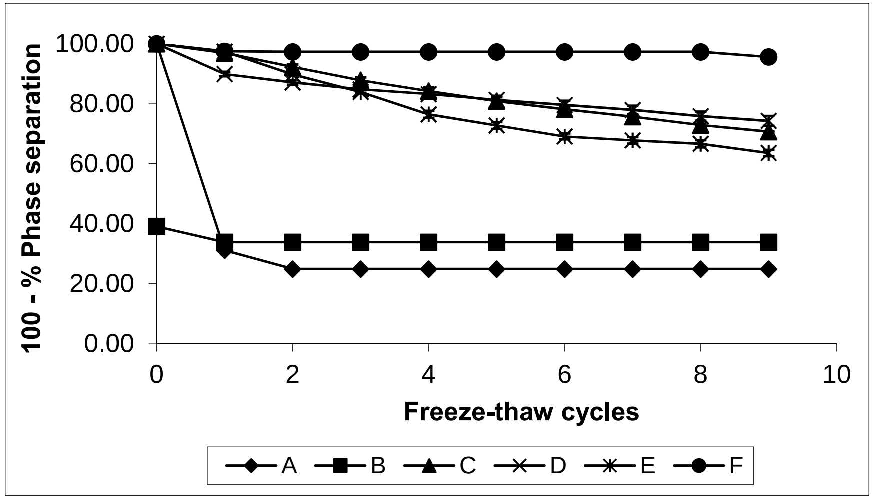
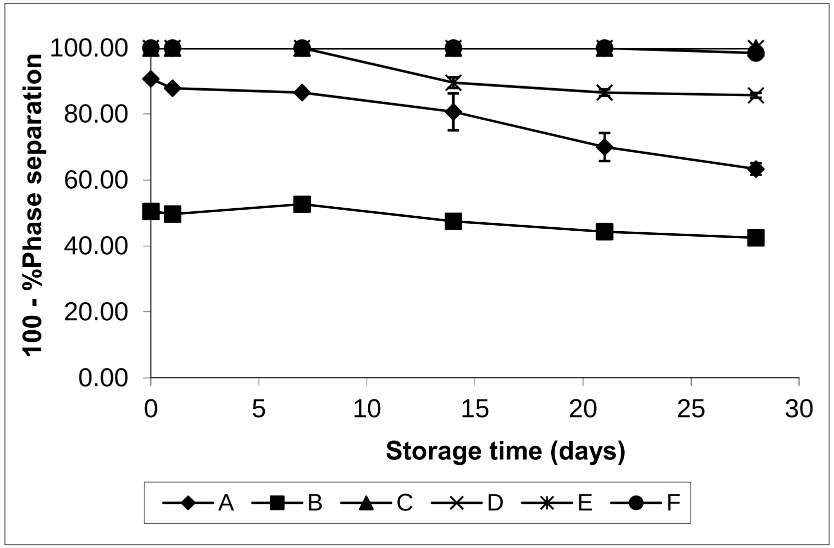


Figure 4.15: 100 - Percent phase separation of: A-full fat mayonnaise, B-50% oil reduced product with no starch added, C-LCMTE with unmodified teff starch, D-LCMTE with teff starch paste modified with 1.5% stearic acid, E-LCMTE with unmodified maize starch and F-LCMTE with maize starch paste modified with 1.5% stearic acid. i) 50% oil replacement level, ii) 80% oil replacement level and iii) 100% oil replacement level.

**** The graphs for full fat mayonnaise and 50% oil reduced product with no starch added were repeated at 80% and 100% oil replacement levels for comparison.***

****The error bars indicate the standard error of the mean.***

i)



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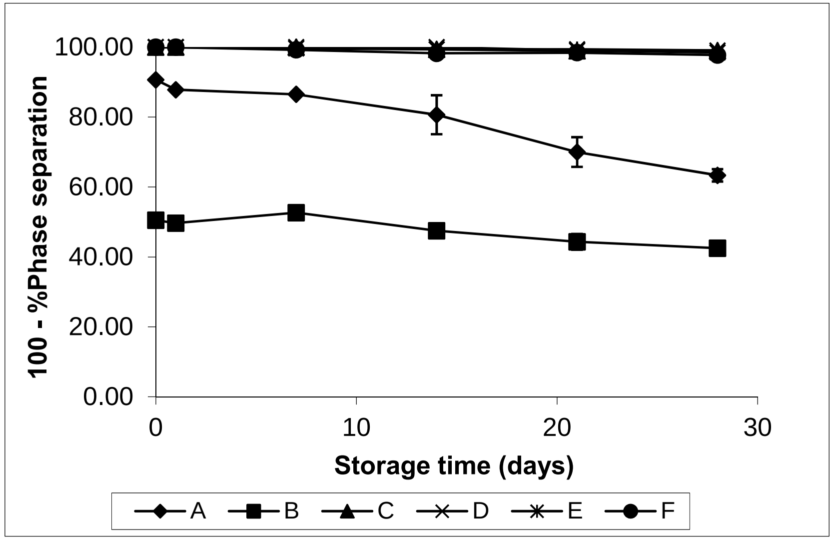

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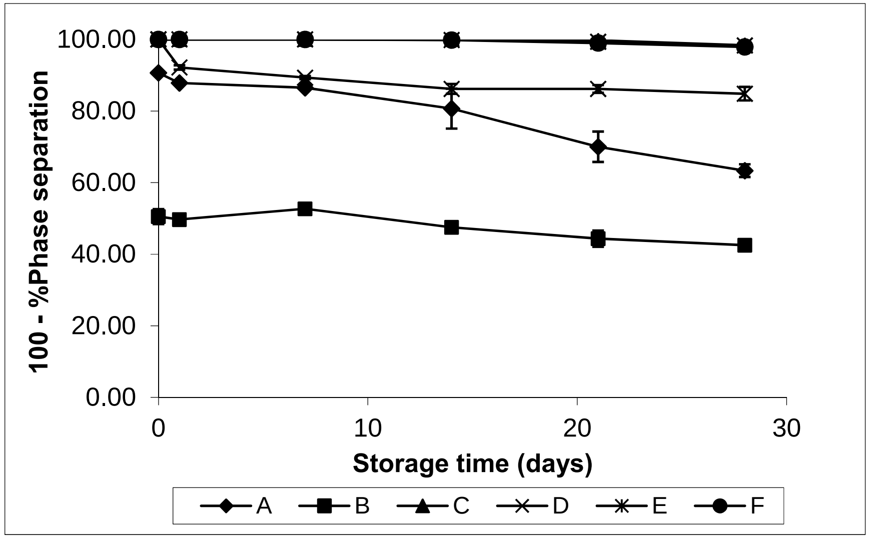

Figure 4.16: 100 - Percent phase separation of: A-full fat mayonnaise, B-50% oil reduced product with no starch added, C-LCMTE with unmodified teff starch, D-LCMTE with teff starch paste modified with 1.5% stearic acid, E-LCMTE with unmodified maize starch and F-LCMTE with maize starch paste modified with 1.5% stearic acid. i) 50% oil replacement level, ii) 80% oil replacement level and iii) 100% oil replacement level.

**** The graphs for full fat mayonnaise and 50% oil reduced product with no starch added were repeated at 80% and 100% oil replacement levels for comparison.***

****The error bars indicate the standard error of the mean***

4.4 Discussion

The difference in protein content between teff and maize starches (Table 4.2) could be due to the extraction methods used. SO_2 is mostly used in the production of commercial maize starch (Kent and Evers, 1994), which reduces the disulphide bonds linking proteins within the protein matrix of the endosperm and promotes solubilization of proteins (Watson, 1984), thus more protein could be separated. On the other hand teff starch was extracted using only distilled water.

As suggested by Richardson et al. (2003) the addition of lipids like stearic acid may lead to the formation of a layer of lipid covering the starch granules which give hydrophobicity to the starch granules. This could have lead to a decreased water uptake and reduced swelling which resulted in no first viscosity peak of teff starch modified with stearic acid (Fgure 4.1). Both D’Silva (2009) and Burger (2010) also found no first viscosity peak for teff starch modified with stearic acid. The second peak viscosity formed for uncomplexed maize starch (Figure 4.1) could be due to formation of complexes between endogenous lipids not removed during defatting and amylose (Nelles et al., 2000). The continuous increase in viscosity with the addition of stearic acid was also found by Bajner (2002) for maize starch and by D’Silva (2009) and Burger (2010) for maize and teff starches. According to D’Silva (2009) addition of stearic acid to maize and teff starch resulted in a second viscosity peak, where the magnitude of the viscosity increased with increase in amount of stearic acid suggesting the formation of amylose-lipid complex to be responsible for the viscosity increase. On the other hand addition of stearic acid to waxy maize starch (virtually no amylose) did not lead to formation of a second peak suggesting that amylose and stearic acid are responsible for the formation of the second peak viscosity. Burger (2010) found X-ray difractograms of teff and maize starches modified with stearic acid (0.25% and 1.5%) to have the characteristic V-type amylose-lipid complex crystallinity peaks. DSC thermograms of the modified starches also showed melting of crystallites typical of amylose-lipid complex (99 – 120 ^{o}C).

Generally LCMTE with maize starch had higher viscous properties with higher yield stress and consistency coefficient (K) values than LCMTE with teff starch (Tables 4.3, 4.4 and 4.5). The possible reason for this could be the higher retrogradation tendency of maize starch compared to teff starch to form a stronger network. Burger (2010) has found maize starch to have higher final viscosity than teff starch after cooling.

Compared to LCMTE with unmodified teff/maize starches, LCMTE with teff/maize starches modified with stearic acid had lower viscous properties as shown by lower yield stress and consistency coefficient (K) values (Tables 4.3, 4.4 and 4.5). This could be because of the properties of the modified paste. D'Silva (2009) found addition of stearic acid to teff and maize starches led to formation of a soft, non-gelling paste while the control teff and maize starch pastes formed a gel upon cooling. The addition of stearic acid may result in the formation of amylose-lipid complex which could hinder the reassociation of amylose molecules.

Compared to the full fat mayonnaise LCMTE with modified and unmodified maize starch at 50%, 80%, and 100% oil replacement levels had higher yield stress and consistency coefficient (K) value (from Herschel-Bulkley or Power Law models) except modified teff (Tables 4.3, 4.4 and 4.5). The possible reason could be the increase in viscosity of the continuous phase. Starch stabilizes substantial amount of water due to its hydrophilic nature and increases viscosity and body (Yackel & Cox, 1992; Glicksman, 1991).

Storage for 8 days at room temperature increased the viscous properties in terms of higher yield stress and consistency coefficient (K) values of LCMTE with unmodified teff and modified and unmodified maize starches (Tables 4.3, 4.4 and 4.5). During storage further retrogradation will occur mainly due to the re-crystallization of the amylopectin fraction (Gudmundsson, 1994) and retrograded starches may have increased the viscosity. LCMTE with teff starch modified with stearic acid behaved differently. For an unknown reason, it did not follow the above trend. The yield stress and consistency coefficient (K) values were lower than the full fat mayonnaise and these values decrease with storage.

The lower shear thinning behaviour (higher n-value) of LCMTE with teff starch compared to LCMTE with maize starch (Tables 4.6 and 4.7) could be due to the higher resistance of teff starch to breakdown compared to maize starch (Bultosa & Taylor, 2004). D'Silva (2009) found maize starch paste to be more shear thinning than teff starch paste. On the other hand the higher shear thinning behaviour of LCMTE with teff/maize starch pastes modified with stearic acid compared to LCMTE with unmodified teff/maize starches could be due to the lubricating effect of stearic acid (Husseini & Pitt, 2008). D'Silva (2009) also found addition of stearic acid to teff and maize starches reduced the n-value compared to the control starch pastes.

The higher stability of teff starch to breakdown compared to maize (Bultosa & Taylor, 2004) could also be the reason why LCMTE with teff starch had lower hysteresis loop area (more stable) than LCMTE with maize starch (Table 4.8). Teff starch modified with stearic acid seems to be a better stabilizer resulting in lower hysteresis loop area compared to unmodified teff and maize starch modified with stearic acid. This could be due to the less gelling tendency of the starch pastes modified with stearic acid compared to unmodified starch pastes (D'Silva, 2009). In LCMTE with unmodified teff/maize starch pastes (which have more gel like structure), an increase in shear rate will break down the gel structure and rebuilding the gel structure will take more time (higher hysteresis loop area). Santipanichwong and Suphantharika (2009) added curdlan to an oil-in-water emulsion and the emulsion had gel-like strength and a very high hysteresis loop area.

The 50% oil reduced product with no starch added had large non-uniform oil droplets aggregated at one area. This could be due to coalescence of oil droplets and phase separation. The very low viscosity of this product (section 4.3.3) could thus be related to its structure. Gutierrez et al. (2002) found that the viscosity of polydisperse (non-uniform) emulsions was significantly lower than that observed in equivalent monodisperse (uniform) emulsions at the same volume fraction. The smaller oil droplets size of LCMTE compared to full fat mayonnaise can also be related to the higher viscosity of the LCMTE than the full fat mayonnaise. For highly concentrated oil-in-water emulsions, the reduction in droplet size results in increase in the viscosity (Pal, 1998).

The storage modulus (G') of the full fat and all LCMTE was larger than the loss molulus (G") (Figure 4.14), throughout the tested frequency range and the tanδ value was less than 1 (Figure 4.15). Thus, it could be suggested that all the samples are weak gel-like. Previous studies found that mayonnaise (full fat or reduced fat with different kinds of fat replacers) exhibited weak gel characteristics in the frequency range of 0.1 -100 Hz (Worrasinchai et al., 2006; Mun et al., 2009). The higher G' value of LCMTE with unmodified teff and maize starches than LCMTE with modified teff and maize starches with stearic acid could be due to the more gelling behaviour of unmodified teff and maize starches than modified teff and maize starches (D'Silva, 2009). Santipanichwong and Suphantharika (2009) studied the effect of different β-glucans on the rheological properties of egg yolk stabilized oil-in-water emulsions and found stronger gels to have higher G' value than the weaker gels. The G' values are related to the yield stress values.

LCMTE with unmodified teff and maize starches had higher yield stress value and smaller droplets compared to LCMTE with modified teff and maize starches with stearic acid. Ma and Barbosa-Canovas (1995) reported an increase in G' with increase in yield stress. Similar relationships between the values of yield stress and storage modulus were also reported by Peressini et al. (1998). Langton et al. (1999) reported that the linear viscoelastic properties as measured by the G' values was found to be more solid-like (higher G') when the mayonnaise was formed of smaller droplets produced by higher energy input during processing, and more liquid-like when it was formed of larger droplets produced by lower energy input. Worrasinchai et al. (2006) also found reduced fat mayonnaise with beta glucan added as fat replacer at 50% and 75% replacement level to contain smaller droplets and having higher G' value than full fat mayonnaise and reduced fat mayonnaise samples at 25% replacement level which contain larger droplets.

A number of physicochemical phenomena that occur during frozen storage may account for the observed influence of freeze–thaw cycling on emulsion stability (Figure 4.16). First, water crystallization occurred when the emulsions were placed in the freezer. As more and more water crystallized the droplets would have been forced closer together (Saito et al., 1999) and there may not have been sufficient free water present to fully hydrate the droplet surfaces (Ausborn et al., 1994; Komatsu, et al., 1997; Strauss & Hauser, 1986), thus favouring droplet–droplet interactions which leads to droplet coalescence and phase separation. Second, ice crystallization leads to an increase in the ionic strength of any freeze-concentrated non-frozen aqueous phase surrounding the emulsion droplets (Komatsu et al., 1997), which may have promoted droplet–droplet interactions which leads to droplet coalescence and phase separation. Third, it is possible that ice crystals formed during freezing may have penetrated into the oil droplets and disrupted their interfacial membranes (Ogawa et al., 2003), thus making them more prone to coalescence. Fourth, cooling may have caused some of the fat in the emulsion droplets to crystallize, which may have promoted partial coalescence due to penetration of fat crystal from one droplet through the membrane of another droplet (Vanapalli et al., 2002; Harada & Yokomizo, 2000). LCMTE with modified and unmodified starches had better freeze-thaw stability than the full fat mayonnaise (Figure 4.16). The possible reasons for this could be the hydrophilic characteristics of the starch pastes may stabilize water from crystallization and the starch pastes could also keep the oil droplets far apart from each other protecting possible droplet coalescence. Thanasukarn et al. (2004) used casein and whey protein as emulsifiers in palm oil-in-water emulsion and found emulsions stabilized with both casein and whey protein were stable to freeze-thaw cycles compared to emulsion stabilized with Tween 20. These differences were attributed to the fact that Tween 20 forms a thin interfacial membrane, whereas the proteins form relatively thick membranes. The non-gelling behaviour of modified teff and maize starches with stearic acid could have allowed the oil droplets to come together and promote droplet-droplet interaction which leads to coalescence and phase separation. At 100% oil replacement level starch syneresis is the possible cause for the destabilization of the LCMTE.

The 50% oil reduced product with no starch added showed higher phase separation after 10 min storage at 50 ^{o}C compared to the full fat mayonnaise (Figure 4.17). This could be because it did not form a shelf stable mayonnaise and phase separation occurred immediately after preparation. The full fat mayonnaise also showed phase separation after 10 min storage at 50 ^{o}C. This could be due to increased motion of oil droplets and solublization of surfactants with increase in temperature which increase the interaction of the droplets and lead to coalescence and phase separation (Depree & Savage, 2001). The LCMTE with modified and unmodified starches were more stable to high temperature storage (lower phase separation) compared to the full fat mayonnaise (Figure 4.17). The possible reason for this could be the addition of the starch pastes which increase the viscosity of the aqueous phase which slows down the droplet motion and could also form a protective layer around the oil droplets protecting the solublization of the surfactant. Mun et al. (2009) used 4αGTase-modified rice starch and xanthan gum in reduced fat mayonnaise and found the reduced fat mayonnaise with 4αGTase-modified rice starch to be more stable than the full fat mayonnaise, which they proposed is due to slow oil droplet movement because of viscosity increase of the aqueous phase by the addition of the starch. Ogawa et al. (2003) added a secondary chitosan layer above the lecithin layer in oil-in-water emulsions and found the emulsion with the secondary layer to be stable to high temperature storage up to 90 ^{o}C.

4.5 Conclusions

LCMTE can be produced with modified and unmodified teff and maize starches, but the properties depend on the level of oil reduction. Modified and unmodified teff and maize starch with stearic acid can produce LCMTE at 50% oil replacement. When the oil content is further decreased to 80% and 100% the LCMTE made with stearic-acid modified starches are the most similar to the full fat product in terms of physical properties. The LCMTE with unmodified starches have non-pourable, gel-like behaviour with higher yield stress and viscosity values than the full fat mayonnaise. The viscosity of the LCMTE increases with storage time except those made with teff starches. The flow properties of LCMTE can be related to their microstructure. The higher viscosity of LCMTE can be related to smaller fat globules and gelling behaviour. All LCMTE are more stable to freeze-thaw cycles and high temperature storage than the full fat mayonnaise. Generally LCMTE can be produced without oil and this can substantially decrease the calorific value of mayonnaises. The reduction in the calorie may contribute to weight loss, and prevention of cardiovascular diseases. The starch pastes modified with stearic acid may also have a potential to be used as emulsifiers because it has both polar and non polar ends due to the stearic acid. If proven efficient to be used as emulsifiers they can replace the egg yolk in emulsions which contains cholesterol.

5. GENERAL DISCUSSION

In this chapter the major results and methodology used in the research project will be critically discussed.

5.1 *Review of Methodology*

The major methods used in the research project are critically reviewed below with respect to short-comings and impact on results.

5.1.1 Starch Pasting

The Rapid Visco Analyser (RVA) was used to prepare the starch pastes to be used in the low-calorie mayonnaise type emulsions (LCMTE). In order to avoid clumping and settling of the starch in the edges of the canister, water was first measured into the canister before the teff and maize starches was added. The starch suspension was then manually stirred using the paddle prior to being placed in the RVA as a precaution to limit clumping and settling which could result in high variability among repetitions. But the manual stirring may not completely eliminate this problem. Thus, only repeatable measurements were taken as reliable data.

The teff and maize starch samples were heated to 91 °C and held at this temperature for 2 hr. The reason for heating to 91 °C and not to 95 °C was to prevent boiling, as water boils at approximately 95 °C in Pretoria because of the high altitude (approximately 1400 m). The RVA in which pasting was conducted is not a closed system and moisture loss could have occurred throughout the long holding time at this high temperature (91 °C). Generally only about 1g of water was lost and this was readded at the end. Water loss could affect the flow properties of the LCMTE.

5.1.2 Flow Property Measurement

The flow properties of the mayonnaise samples were determined using a rheometer at varying shear rates of 0.01 s^{-1}–1000 s^{-1}. The measuring system used was the bob and cup method (a measuring bob inserted into a cup containing the sample). Mayonnaise is a shear thinning material where the viscosity decreases with increase in shear rate. In emulsions like mayonnaise a large decrease in viscosity is observed above a critical shear rate (Barnes, 1994; Otsubo &

Prud'homme, 1994). When wall slip occurs, this drop in viscosity is seen at a lower shear rate value. Apparent wall slip occurs in emulsions when the dispersed phase migrates away from solid boundaries, like the walls of the sensor system in a rheometer, leaving a thin layer of lower-viscosity liquid. This leads to underestimation of the viscosity. Bower et al. (1999) used an optical microscope to observe the shearing process in mayonnaise. They observed a slippage only at shear rates below 1 s^{-1}. In this study, the vane system and plate-plate measuring system at different gaps were also used to determine the slip effect on the full fat mayonnaise sample. Vane method was used because it eliminates slip since shearing occurs completely within the material along the localized surface circumscribed by the vane (Dzuy & Boger, 1983). The viscosity results from the vane system were not significantly different from the viscosity values from the bob and cup method (Table 5.1) indicating that there was no wall slip in the bob and cup method at shear rates above 1 s^{-1}. The flow property values considered for this study are also those above the shear rate of 1 s^{-1}. The reason why the vane system was not used for measuring the flow properties and viscoelastic behaviour was because it was not available when the experiment was conducted. The plate-plate geometry at different gaps was also used to check the wall slip. If the viscosity values measured at different gaps are similar it indicates that there is no wall slip (Campbell et al., 2005). If the viscosity values measured at different gaps differ it indicates the presence of wall slip (Durairaj et al., 2009). As shown in Table 5.1 at shear rates above 1 s^{-1} the viscosity values at different gaps were similar indicating the absence of wall slip at shear rates above 1 s^{-1}, the values considered for this study.

Table 5.1 Viscosity value comparisons of full fat mayonnaise with different measuring system.

Shear rate (s^{-1})	**Viscosity (Pa.s)**					
	Bob and cup	Vane	Plate-plate (at different gaps)			
			0.5 mm	1 mm	1.5 mm	2 mm
0.01	444	396	498	500	529	499
1	29.2	32.8	20.3	28	26.2	25.8
10	4.88	5.07	4.58	4.78	4.6	4.47
100	1.07	1.05	1.01	1.0	0.97	0.94
1000	0.24	0.30	0.26	0.24	0.23	0.001

Thus it seems that no wall slip occurred in the bob and cup method and the flow property values presented in this study are most probably real values of the mayonnaise samples.

5.1.3 Viscoelastic behaviour measurement

To investigate the structural properties of mayonnaise samples small amplitude shear tests were conducted within the linear viscoelastic region. This small amplitude oscillatory experiment has the advantage of minimizing destruction in the sample compared to the steady state flow measurement which is a result of progressive structural destruction (Worrasinchai et al., 2006). Relationship between the complex viscosity from the small amplitude oscillatory tests and the steady shear viscosity of the full fat mayonnaise and low-calorie mayonnaise type emulsions was established (Figure 5.1). The steady shear (large deformation) viscosity (η) of all mayonnaise samples was apparently lower than the complex viscosity (η*) (small deformation). This suggests that mayonnaise behaviour was not described by the Cox–Merz rule, and this phenomenon is typical of weak gels (Richardson et al., 1989).

The Cox/Merz relation is an empirical relationship that equates the shear rate dependence of the steady shear viscosity (η) to the frequency dependency of the complex viscosity (η*) at equal values of shear rate and angular frequency, that is $\eta(\dot{\gamma}) = \eta^*(\omega)$ where $\dot{\gamma} = \omega$. This relation provides insight on the structure of the sample. Many polymer melts and solutions, obey the Cox/Merz rule. But any kind of physical and/or chemical interactions lead to a certain deviation from the Cox/Merz relation and therefore, this relation is not useful for materials showing G' > G'' (gel character) in the low-shear range like stable dispersions (suspensions, emulsions, foams), pastes and gels (Mezger, 2006).

Worrasinchi et al. (2006) also reported that mayonnaise is not described by the Cox/Merz rule. Santipanichwong and Suphantharika (2009) found similar results for egg yolk stabilized oil-in-water emulsions with different β-glucans.

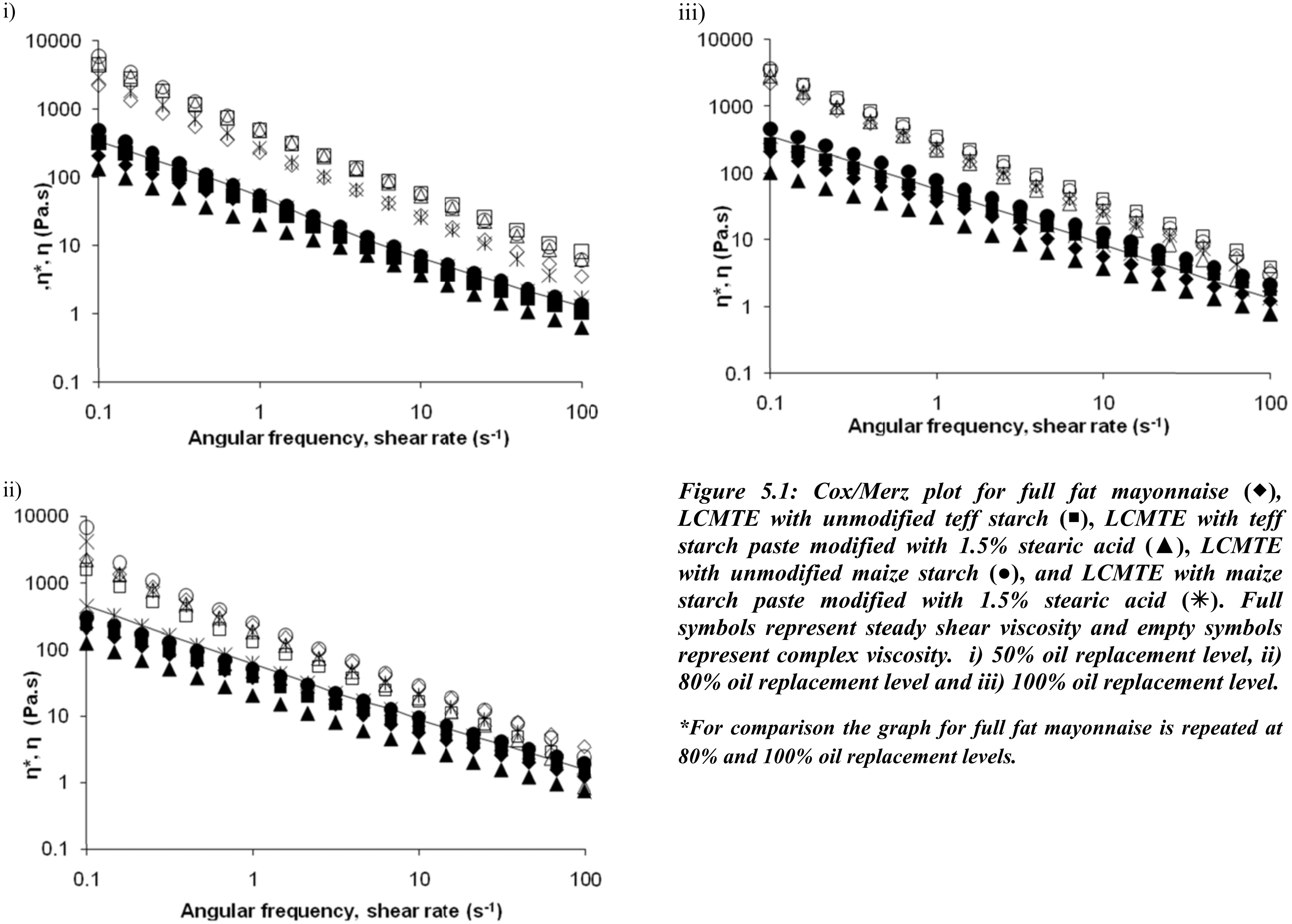

***Figure 5.1:** Cox/Merz plot for full fat mayonnaise (◆), LCMTE with unmodified teff starch (■), LCMTE with teff starch paste modified with 1.5% stearic acid (▲), LCMTE with unmodified maize starch (●), and LCMTE with maize starch paste modified with 1.5% stearic acid (✳). Full symbols represent steady shear viscosity and empty symbols represent complex viscosity. i) 50% oil replacement level, ii) 80% oil replacement level and iii) 100% oil replacement level.*

**For comparison the graph for full fat mayonnaise is repeated at 80% and 100% oil replacement levels.*

5.1.4 Microscopy

Optical microscopy and confocal laser scanning microscopy (CLSM) were used to determine the effect of teff and maize starch pastes (unmodified and modified with stearic acid) added to replace the oil on the microstructure of the low-calorie mayonnaise type emulsions. Mun et al. (2009) and Izidoro et al. (2009) used optical microscopy to observe the microstructure of full fat and reduced fat mayonnaise samples with modified rice starch and green banana pulp respectively. Both sets of authors found mayonnaise samples to consist of oil droplets dispersed in aqueous medium. The results for this study were also in agreement with these results. The CLSM also showed similar results with the optical microscopy but the images from the CLSM were clearer. CLSM, a relatively new technique used to study microstructure of food products (Van de Velde et al., 2003), has several advantages over conventional optical microscopy (Minsky, 1998). The advantages include: elimination of out-of-focus images by having a confocal pinhole thus producing a better resolution in the lateral as well as axial directions, and its ability to deliver in focus images by true means of depth-wise slicing (Park & Kihm, 2006). Additionally CLSM enables samples to be observed without major preparation procedures (Heertje et al., 1987). CLSM forms a bridge between conventional light microscopy, with its ability to image hydrated live specimens but with limited resolution, and electron microscopy, with its greater resolution but need for extensive specimen preparation (Brankenhoff et al., 1988). CLSM was used by different authors to characterize the microstructure of mayonnaise samples (Langton et al., 1999; Santipanichwong and Suphantharika, 2009). Hassan et al. (1995) also found CLSM to be a useful technique to visualize the formation and three-dimensional microstructure of yoghurt gels in their natural fully hydrated state. The size of the oil droplets for the full fat mayonnaise found in this study were generally larger (5-10 µm) compared to the results of other authors which ranges from 2 µm to 5 µm (Santipanichwong and Suphantharika, 2007; Mun et al., 2009). Even though the CLSM enables one to clearly see the oil droplets, the proposed amylose-stearic acid complexes in the LCMTE with starches modified with stearic acid were not visible. To visualize the complexes the stearic acid could be labelled with fluorescent indicator molecules such as fluorescein, nanoparticles of lanthanide oxides before its addition to the starch so that the complexes could be tracked by the indicator molecules. Nanoparticles of lanthanide oxides are promising labels in biotechnology because of their optical properties such as large stokes shift, lack of photoleaching and long fluorescence lifetime (Bhargava, 1996; Tissue, 1998)

5.2 *The effect of teff and maize starch pastes (unmodified and modified with stearic acid) on the flow properties and microstructure of low-calorie mayonnaise type emulsions (LCMTE)*

Table 5.2: A comparative summary of the overall effects of starch type, modification, increasing level of oil replacement and storage on the flow properties and microstructure (oil droplet size) of low-calorie mayonnaise type emulsions (LCMTE).

	Yield stress	K-value	n-value (Herschel-Bulkley)	n-value (Power Law)	Oil droplet size
Starch type	Maize higher than teff	Maize higher than teff	No significant effect	Maize lower than teff	Maize smaller than teff
Starch Modification	Decreased	Decreased	No significant effect except a decrease for modified teff	No significant effect	Increased
Increasing level of oil replacement	Decreased from 50% to 80% and increased when increased to 100% except modified teff	Increased except for modified teff starch in Power Law model	No significant effect	No significant effect except for unmodified teff	No clear effect was observed
Storage for 8 days	Increased except modified teff at 80% and 100% and modified maize at 50%	Increased except for modified teff starch at 80% and 100%	Decreased except for modified teff starch	Decreased except for modified teff at 80% and 100%	Increased only for modified teff starch,

LCMTE with unmodified teff and maize starch pastes were found to have higher yield stress and viscosity values compared to the full fat mayonnaise. The LCMTE with unmodified teff and maize starches were also found to be non-pourable. Thus, the unmodified teff and maize starch pastes seem to act only as stabilizers. On the other hand LCMTE with modified starches were found to have similar flow properties and microstructure to the full fat mayonnaise. They were also found to be pourable like the full fat mayonnaise. Hence, LCMTE with modified starches could replace the full fat mayonnaise, that is teff and maize starch pastes modified with stearic acid (1.5%) can be used as fat replacer in low-calorie mayonnaise type emulsions replacing up to 100% of the oil. The non gelling behaviour of the modified teff and maize starch pastes was believed to be due to the formation of amylose-stearic acid complexes (D'Silva, 2009). These modified starch pastes seem to have polar and non-polar ends due to the stearic acid. Therefore, in addition to their use as fat replacer the teff and maize starches modified with stearic acid could be used as emulsifiers to replace the egg yolk. In emulsions like mayonnaise which use egg yolk as an emulsifier cholesterol (found in the egg yolk) is a concern for health. Thus, the amylose-stearic acid complexes could be a good alternative to the egg yolk as emulsifier and future works need to investigate this. The amylose-lipid complexes may also act as undigestible starch which further reduces the calorie count (which has health benefits). The LCMTE could be used as low calorie (Table 5.3) alternatives for health conscious consumers. They could have a potential benefit in weight loss; reduce the risk of cardiovascular diseases and some types of cancer. Pre-gelatinised teff and maize starches modified with stearic acid (1.5%) could be produced, with holding time of 2 hrs, and they can be used to produce LCMTE at large scale.

Table 5.3: Chemical composition (%, w/w) and caloric values of mayonnaise samples

Sample	Fat	Carbohydrate	Protein	Ash	Moisture	Caloric values (Kcal/100 g)*
Full fat Mayonnaise	73.78	1	1.92	0.21	23.09	675.7
LCMTE 50% oil replacement	38.78	4.5	1.92	0.22	54.58	374.7
LCMTE 80% oil replacement	17.78	6.6	1.92	0.22	73.48	194.1
LCMTE 100% oil replacement	3.78	8	1.92	0.23	86.07	73.7

These are computed values presented only to give an indication how much calories are lowered

*Caloric values = (9×fat) + (4×protein) + (4×carbohydrate)

6. CONCLUSIONS AND RECOMMENDATIONS

Teff and maize starch pastes (with holding time of 2 hr) modified with stearic acid (1.5%) can be a fat replacer ingredient to produce mayonnaise type emulsion with no oil. Unmodified teff and maize starches can be used as fat replacer at 50% oil replacement in LCMTE, but not at 80 and 100% oil replacement. Unmodified teff and maize starches result in LCMTE with higher yield stress and viscosity values compared to full fat mayonnaise probably because of smaller size oil droplets compared to full fat mayonnaise and LCMTE with modified teff and maize starches. The smaller size oil droplets can therfore be related to the higher yield stress and viscosity values of the LCMTE with unmodified starches. The lower yield stress and viscosity values of LCMTE with modified teff and maize starches compared to LCMTE with unmodified starches can be due to the non-gelling behaviour of the modified starch pastes which is believed to be due to the formation of amylose-stearic acid complexes. Amylose-stearic acid complexes have a role to play in the production of LCMTE with no oil, but optical and/or confocal laser scanning microscopy cannot confirm this. Thus, labelling the stearic acid with indicator molecules may enable tracking of the amylose-stearic acid complexes.

The LCMTE with teff and maize starches modified with stearic acid could be potentially used as low-calorie alternatives of mayonnaise for health-conscious consumers. The reduction in calories may contribute to weight loss, and prevention of cardiovascular diseases. The starch pastes modified with stearic acid may also have a potential to be used as emulsifiers because it has both polar and non polar ends due to the stearic acid. If proven efficient to be used as emulsifiers they can replace the egg yolk in emulsions which contains cholesterol.

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