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EFFECT OF LOW AND HIGH MOLECULAR WEIGHTS WATER SOLUBLE CHITOSAN ON OXIDATION OF WHOLE MILK POWDER

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EFFECT OF LOW AND HIGH MOLECULAR WEIGHTS WATER SOLUBLE CHITOSAN ON OXIDATION OF WHOLE MILK POWDER

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ABSTRACT

Autoxidation significantly decreases the shelf life of whole milk powder due to primary and secondary oxidation products such as hydroperoxides, aldehydes, ketones, alcohols and hydrocarbons. Water soluble chitosan has been shown to reduce oxidation by chelating metals or combining with lipids resulting in a significant antioxidative effect. The objective of this study was to determine the antioxidative effects of different concentrations of low (L) and high (H) molecular weight water soluble chitosan (9 and 90 kDa, respectively) on whole milk powder (WMP). Commercially dried WMP was obtained and rehydrated 50% (wt/wt) with various aqueous chitosan solutions (2% L2, 4% L4, 2% H2, 4% H4) (L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added) yielding approximately 50% solids concentration. A control was prepared with no added chitosan (0%). Rehydrated WMP was freeze-dried then all samples were stored at 45°C for 48 days. Samples were evaluated for moisture content, water activity, color, thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) every 8 days for 48 days. Moisture and water activity of control, L2, L4, H2, and H4 treatments did not significantly differ during storage. The addition of chitosan tended to result in lower L*, H and higher a* values. The b* values were also higher in WMP with added chitosan near the end of storage. C* values for H2 and H4 were higher than other

treatments. TBARS of L2, H2 and H4 were not significantly different from control TBARS during storage except the TBARS of H2 and H4 which were higher than control TBARS on days 40 and 48 ($P<0.05$). PV of L2, H2, H4 was significantly lower than control PV on days 16, 24, 32 ($P<0.05$). TBARS and PV of L4 were significantly lower than control values on days 0, 16, 40 and 48 ($P<0.05$). All treatments of chitosan exhibited a significant increase in TBARS and PV ($P<0.05$) values during storage. However, 4% of the low molecular weight chitosan inhibited oxidation as measured by TBARS and PV when compared to all other treatments and the control.

DEDICATION

I would like to dedicate this work to my ever dearest father and mother who have induced me to study harder. Also, I would like to thank my brothers and sister for always believing in me. Without them I would not be the strong independent man that I am.

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TABLE OF CONTENTS

Table of Contents (Continued)

Page

LIST OF TABLES

Page

List of Tables (Continued)

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

The demand for whole milk powder (WMP) has increased during recent years [1], due to its nutritional value (high-quality protein, high in soluble vitamins and minerals including calcium, phosphorus and magnesium), and resistance to microbial growth [2, 3]. The average composition of WMP is protein 26.5%, lactose 38.0%, fat 26.75%, total minerals 6.0%, and moisture 2.25% (Table 1.1).

Table 1.1. Typical composition of whole milk powder

No	Ingredients	Total amounts (%)
1	Protein	26.5
2	Lactose	38.0
3	Fat	26.75
4	Total Minerals	6.0
5	Moisture	2.25

From US. Dairy Export Council, 2011[28].

Whole milk powder (WMP) has become an popular product worldwide with consumption of WMP at 23,000, 49,000, 90,000, 362,000, and 1,489,000 metric

tons in the U.S, Australia, Argentina, European Union and China, respectively in 2012 [1]. However, dehydration can make milk more susceptible to lipid oxidation [4–8]. Milk lipid oxidation occurs through a free radical mediated chain reaction [9, 10], limiting the shelf life of WMP to approximately 6 months [11]. The 26−28.5% fat content of WMP is high in polyunsaturated fatty acids which oxidize rapidly during storage [12–15]. Antioxidants are substances added to food to extend shelf life by delaying the onset of oxidation and reducing fat rancidity [16, 17]. Antioxidants scavenge free radicals and inhibit other oxidative reactions [18–20]. Recently, the antioxidant activity of chitosan has received attention with reports finding the antioxidant activity of low molecular weight water soluble chitosan being similar to α−tocopherol [21–23]. Water soluble chitosans will chelate metals in addition to exhibiting a free-radical scavenging effect in the presence of lipids [24]. Incorporation of 0.2%, 0.5% and 1% chitosan of various molecular weights (30, 90 and 120 kDa) into salmon was found to retard lipid oxidation [25]. Low molecular weight chitosan (LMWC), which has high water solubility, may be considered a potential natural antioxidant for stabilizing lipid−containing foods to prolong shelf life [25]. A pH below 6.0 was found to be optimal for achieving desirable antimicrobial and antioxidative-preservative effects with low molecular weight chitosan in liquid and solid foods [26]. Chitosan at low pH (<0.6) inhibited the growth of spoilage organisms in chickpeas and LMWC had higher scavenging activity in apple juice than high molecular weight chitosan (HMWC) [26]. Furthermore, coating bread with 2% high molecular weight chitosan lowered

thiobarbituric acid-reactive substances (TBARS) during storage [27]. Thus, the use of water soluble chitosans with WMP may benefit the dairy industry and other food manufacturers that use WMP as an ingredient by limiting oxidation and extending the shelf life.

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RESEARCH OBJECTIVE

The objective of this study was to determine the antioxidant effects of low and high molecular weight water soluble chitosans on the stability of whole milk powder.

CHAPTER II

LITERATURE REVIEW

Whole Milk Powder Functional Properties

Whole milk powder (WMP) is an important ingredient used in several foods to improve quality and appeal by increasing functional properties such as water binding, solubility, emulsification, viscosity, whipping/foaming, gelation and heat stability to many foods [1, 4]. WMP is produced by removing water from pasteurized, homogenized whole milk [2, 3]. WMP is used for reconstitution and can be used as a food ingredient in "value-added foods" such as confectionery, bakery, and meat products [4]. The proximate composition of WMP is protein 26.5%, fat 26.75%, lactose 38.0%, with trace percentages of vitamins, calcium, phosphorus, and magnesium which contributes to the nutritional quality of milk [2, 3]. WMP also contains fat which consists of essential fatty acids such as linoleic and linolenic acid. Furthermore, it creates a smooth creamy texture in baked goods, confections, meat, and other prepared foods [2, 3]. Moreover, WMP has a longer shelf life than liquid milk due to its low moisture content (2.0%−4.5%). In addition, WMP does not require refrigeration. The Iraqi people consume in the range of 120,000 to 200,000 tons of milk powder per year [36], making a critical food staple in that country.

Chitosan

Chitosan is polysaccharide readily found in nature in marine shells and other sea crustaceans. Chitosan has widespread application in the pharmacological, biomedical and agriculture industries. Chitosan has received attention as an antimicrobial, antioxidant, immunity-enhancer, antitumor, anticancer, and anti-inflammatory agent, due to its biocompatibility and low toxicity [5, 6]. It is a polysaccharide extracted from the shells of crustaceans, such as shrimp, crab and other sea crustaceans. Chitosan is chemically named 2-amino-2-deoxy-b-D-glucopyranose with molecular formula of $(C_6H_{11}O_4N)n$ [7]. Chitin has two hydroxyl groups in its structure while chitosan has one amino group and two hydroxyl groups (Figure 2.1).

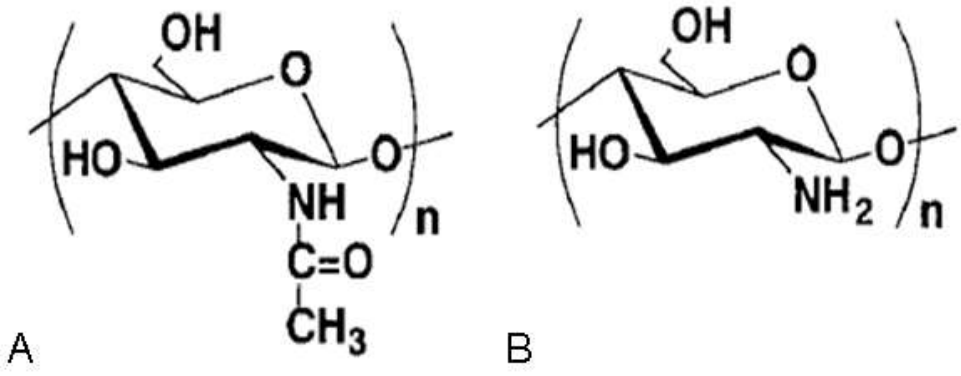

Figure 2.1: Chemical structures of chitin (A) and chitosan (B) [8]

Chitosan can behave like a fiber giving it unique properties including the capacity to be formed into films and non−toxic polymers. In the last several years,

chitosan has been studied as a renewable polymer with applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification. Chitosan has also been used in applications for wastewater purification treatments, and for food functionality such as binding, gelling, thickening and stabilizing [9].

Characteristics of Chitosan

Chitosan is structurally similar to cellulose in the chemical structure (Figure 2.2).

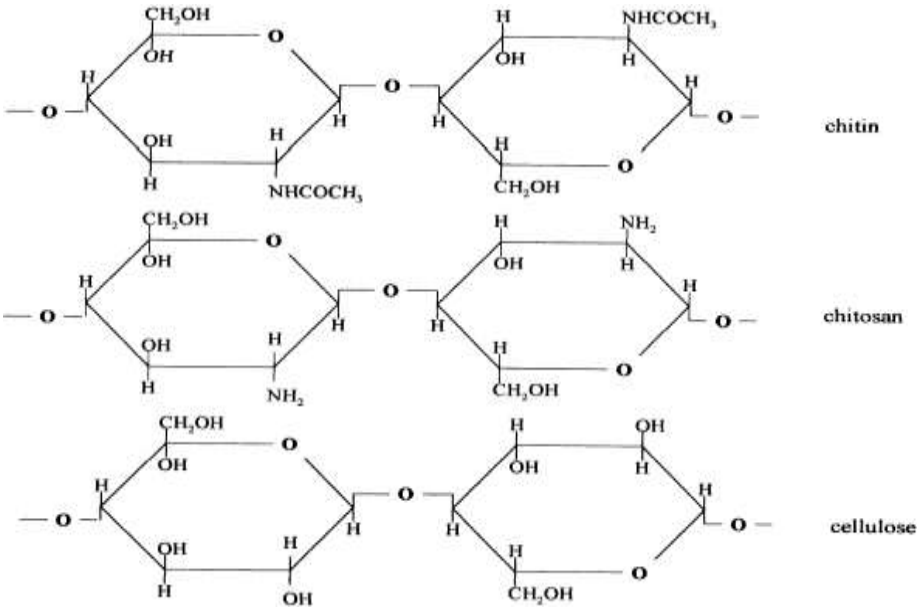

Figure 2.2: structure of chitin, chitosan, cellulose [10]

The difference between chitosan and cellulose is that chitosan contains the amine (−NH2) group in the position C−2 while cellulose has a hydroxyl (−OH) group (Figure 2.2).

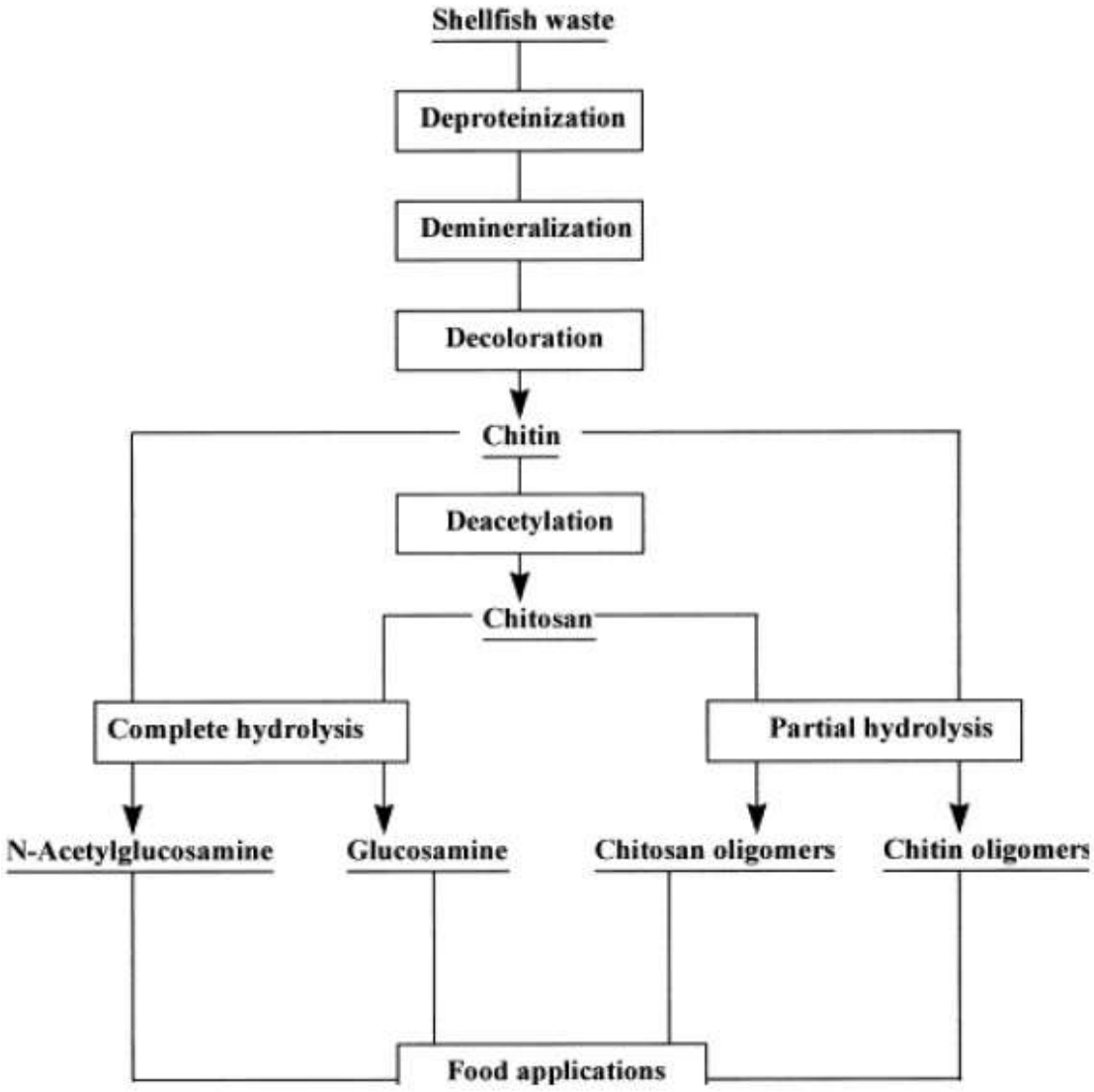

Figure 2.3: Flowchart for preparation of chitin, chitosan, associated oligomers and monomers from shellfish waste [11].

Demineralization and deproteination of shellfish waste can be accomplished using dilute hydrochloric acid to remove metals and salts and exposure to an alkaline medium to hydrolyse the protein and pigments. Chitin is then deacetylated and dried to yield chitosan (Figure 2.3). A major step to produce chitosan requires the alkaline deacetylation of chitin with strong alkaline solution [20]. Chitosan has three types of reactive functional groups which are an amino group at the C-2 position and two hydroxyl groups at the C−3 and C−6 positions. The chemical modification of the amino group and hydroxyl groups have provided multiple useful materials for different applications [11] (Figure 2.3).

Chitosan contains positive ionic charges, which gives it the capacity to chemically bind with negatively charged lipids, cholesterol, metal ions, proteins, and macromolecules [12]. Chitosan has received increasing commercial interest as a resource material due to its biocompatibility, biodegradability, solubility, antioxidant properties and its ability to form complexes with transition metals, and to chelate metal ions [13, 14].

Sources of Chitosan

Seafood processing waste products such as crab and shrimp shells are excellent commercial sources of chitosan [15]. Some researchers have also extracted chitosan from fungi using alkaline and acid treatments [16, 17]. Other researchers have used microorganisms or proteolytic enzymes for the deproteinization of crustacean chitin wastes to produce chitosan [18, 19].

Lipid Oxidation

Whole milk powder (WMP) contains protein, fat, lactose and other mineral constituents. Long term storage of whole milk powder results in undesirable quality changes including protein deterioration, lactose crystallization (caking), loss of nutrients, lipid oxidation and off-flavor development [1]. Lipid oxidation in whole milk powder is of paramount importance because it results in loss of nutritional value and development of undesirable flavors [21]. External factors affecting the shelf life of whole milk powder include temperature, storage time and an increase in water activity [1, 22]. Unsaturated fatty acids (RH) in whole milk powder are susceptible to oxygen attack which leads to lipid oxidation. Lipid oxidation involves free radical reactions with initiation, propagation, and termination phases. In the initiation phase of lipid oxidation, the reaction of oxygen with unsaturated fatty acids (RH) forms free radical by removing a labile hydrogen from a carbon atom (Figure 2.4). In the propagation phase, free radicals react with unsaturated fatty acids (RH) to produce hydroperoxides (ROOH) leading to the formation of secondary reaction products such as alkanes, alkenes, aldehydes and ketones resulting in off flavors [23] (Figure 2.4). There are other factors promoting lipid oxidation such as light, radiation, and metal ions [24, 25]. Furthermore, some studies reported that seasonal variations in animal feed impact WMP quality. Biolatto et al. [37] reported that seasonal changes impact dimethyl sulphide, n-pentanal, n-hexanal. WMP produced in summer had higher amounts of n-hexanal, n-pentanal and dimethyl sulphide than autumn and winter. Urbach [38] found that

WMP flavors can be affected by animal feed. A poor quality silage and different weeds yield off-flavors in the milk [38].

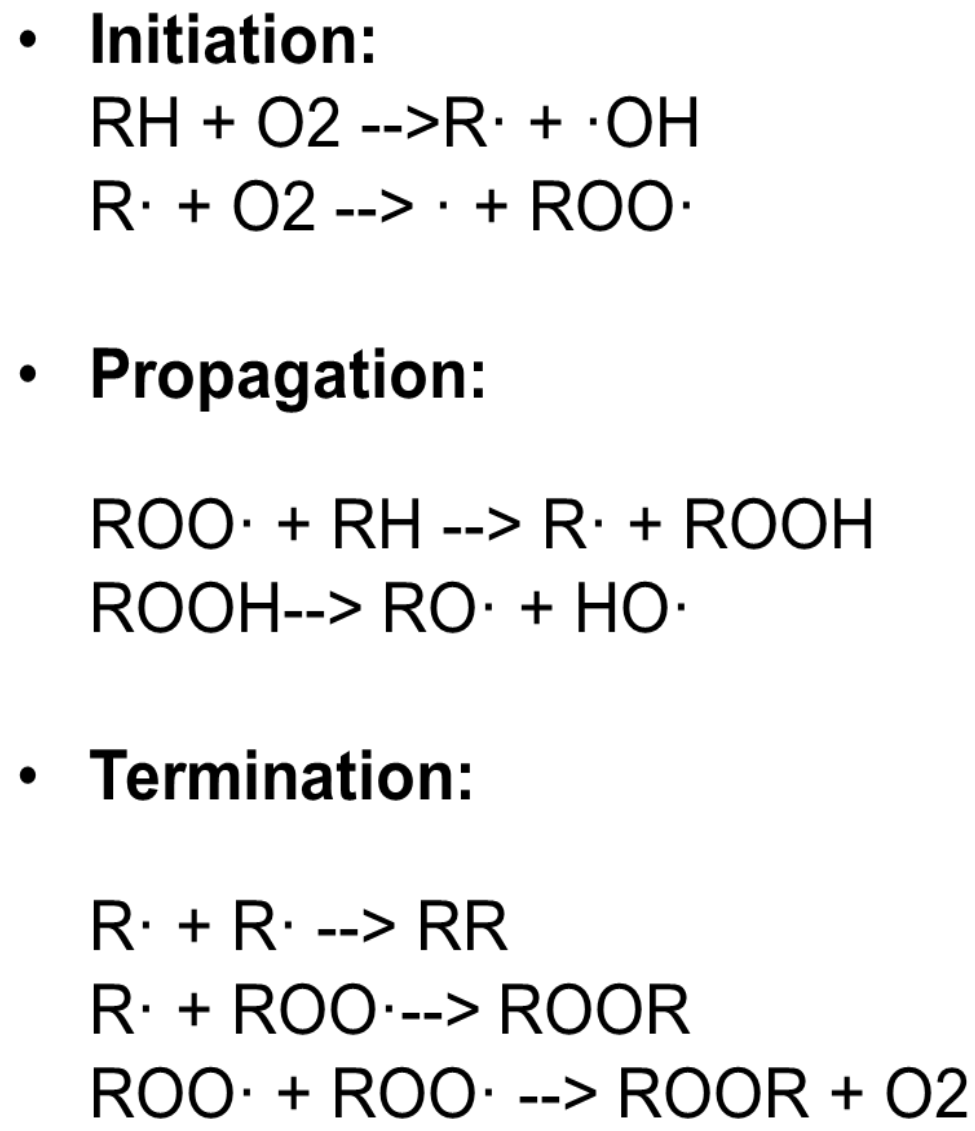

Figure 2.4: Oxidation reactions [26]

Antioxidants Activity of Chitosan

Numerous studies reported that chitosan has antioxidant activity [13, 29, 30, 31]. Park et al. [27] reported that chitosan can exert a strong and equivalent activity to that of phenolic antioxidants. A study by Yen et al. [28] found that chitosan from shiitake mushrooms had antioxidant activity confirmed by measuring reducing power, DPPH (2,2−diphenyl-1−picrylhydrazyl) scavenging, and hydroxyl radical scavenging ability. Feng et al. [29] found that water soluble chitosan was an ideal natural antioxidant, and its antioxidant activity depended on its molecular weight. In addition, total antioxidant activity of ½ N-acetylated water soluble chitosan was similar to that of α-tocopherol [29]. Scavenging activity of low molecular weight ½ N−acetylated water soluble chitosan against superoxide radical was more pronounced than that of high molecular weight ½ N−acetylated chitosan [29]. The scavenging of hydroxyl radicals by chitosan inhibited lipid peroxidation of phosphatidylcholine and linoleate liposomes [30, 31]. Darmadji and Izumimoto [13] observed that addition of 1.0% chitosan to beef decreased the TBA value by approximately 70% compared to that of the control samples after 3 days of storage at 4°C. Chitosan showed consistent antioxidant activity with increased concentration [32] (Figure 2.5).

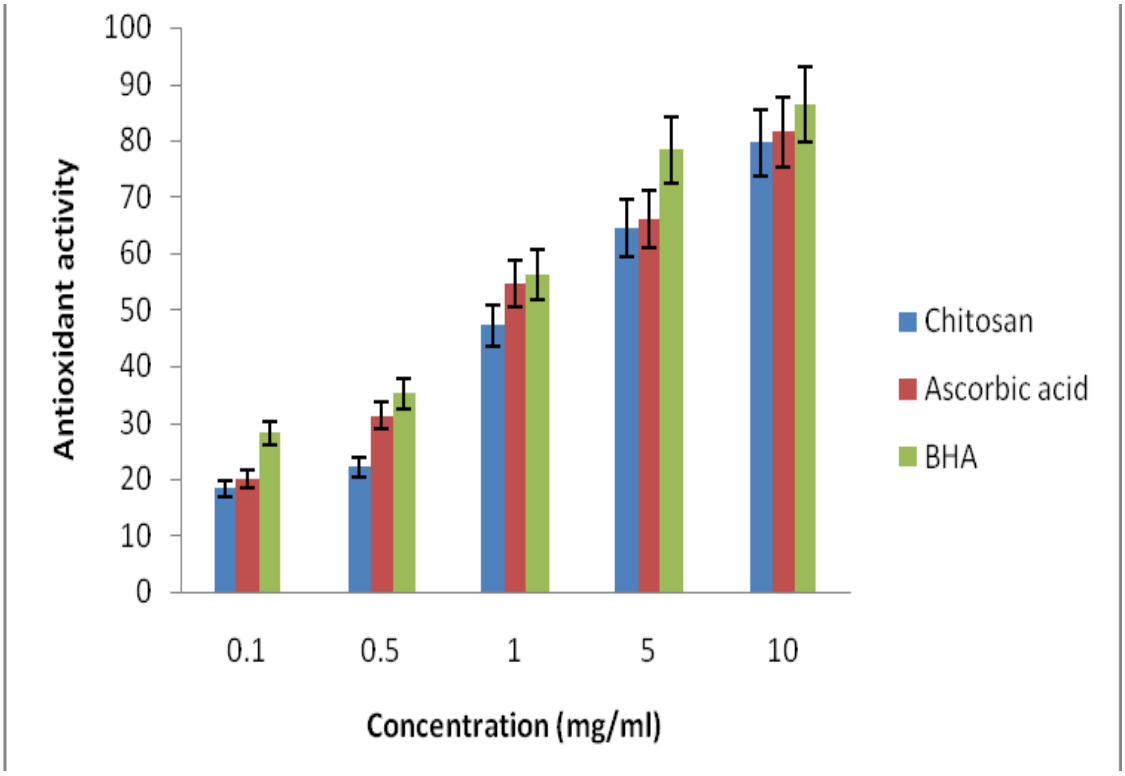

Figure 2.5: Scavenging effects of the chitosan (0.1 to 10mg/ml) from sentinel crab, ascorbic acid and BHA on total antioxidant activity [32].

Other research has shown a relationship between the nitrogen content or amine groups within chitosan and its antioxidant capacity. A study by Park et al. [27] reported that chitosan could scavenge free radicals by nitrogen located on the C-2 position of the chitosan structure. Another study conducted by Xie et al. [31] reported that chitosan has high scavenging activity by producing a stable molecule when the free radicals react with the hydrogen ion from ammonium ions (NH_3^+) of

chitosan. Kim and Thomas [33] reported that low molecular weight chitosan has higher scavenging activity than high molecular weight chitosan and this is likely due to the compact structure of HMWC. The HMWC intramolecular hydrogen bonding is stronger than that of LMWC which suggests that exposure of HMWC amine groups might be restricted [33]. Increased scavenging activity of higher concentrations of LMWC was likely due to the increased presence of reactive amine groups [33]. Another antioxidant activity mechanism of chitosan is generally attributed to chelation [34, 35] since chitosan binds metal ions to prevent initiation of lipid oxidation.

Previous research has shown that low molecular weight chitosan has high antioxidant capacity making it an ideal food additive for foods where lipid oxidation is the primary mechanism for spoilage. However, very little research has been published on the antioxidant activities of chitosan, specifically on the use of chitosan in milk products. Thus, the use of water soluble chitosans with WMP may benefit the dairy industry by limiting oxidation.

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CHAPTER III

EFFECT OF LOW AND HIGH MOLECULAR WEIGHTS WATER SOLUBLE CHITOSAN ON OXIDATION OF WHOLE MILK POWDER

ABSTRACT

Autoxidation significantly decreases the shelf life of whole milk powder due to primary and secondary oxidation products such as hydroperoxides, aldehydes, ketones, alcohols and hydrocarbons. Water soluble chitosan has been shown to reduce oxidation by chelating metals or combining with lipids resulting in a significant antioxidative effect. The objective of this study was to determine the antioxidative effects of different concentrations of low (L) and high (H) molecular weight water soluble chitosan (9 and 90 kDa, respectively) on whole milk powder (WMP). Commercially dried WMP was obtained and rehydrated 50% (wt/wt) with various aqueous chitosan solutions (2% L2, 4% L4, 2% H2, 4% H4) (L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added) yielding approximately 50% solids concentration. A control was prepared with no added chitosan (0%). Rehydrated WMP was freeze-dried then all samples were stored at 45°C for 48 days. Samples were evaluated for moisture content, water activity, color, thiobarbituric acid

reactive substances (TBARS) and peroxide value (PV) every 8 days for 48 days. Moisture and water activity of control, L2, L4, H2, and H4 treatments did not significantly differ during storage. The addition of chitosan tended to result in lower L*, H and higher a* values. The b* values were also higher in WMP with added chitosan near the end of storage. C* values for H2 and H4 were higher than other treatments. TBARS of L2, H2 and H4 were not significantly different from control TBARS during storage except the TBARS of H2 and H4 which were higher than control TBARS on days 40 and 48 ($P<0.05$). PV of L2, H2, H4 was significantly lower than control PV on days 16, 24, 32 ($P<0.05$). TBARS and PV of L4 were significantly lower than control values on days 0, 16, 40 and 48 ($P<0.05$). All treatments of chitosan exhibited a significant increase in TBARS and PV ($P<0.05$) values during storage. However, 4% of the low molecular weight chitosan inhibited oxidation as measured by TBARS and PV when compared to all other treatments and the control.

INTRODUCTION

The demand for whole milk powder (WMP) has increased during recent years [1], due to its nutritional value (high−quality protein, high in soluble vitamins and minerals including calcium, phosphorus and magnesium), and resistance to microbial growth [2, 3]. The average composition of WMP is protein 26.5%, lactose 38.00%, fat 26.75%, minerals 6.00%, and moisture 2.25% [4]. Whole milk powder

(WMP) has become a popular food worldwide with consumption of WMP at 23,000, 49,000, 90,000, 362,000, and 1,489,000 metric tons in the U.S, Australia, Argentina, European Union and China, respectively in 2012 [1]. However, dehydration can make milk more susceptible to lipid oxidation [5–9]. Milk lipid oxidation occurs through a free radical mediated chain reaction [10, 11], limiting the shelf life of WMP to approximately 6 months [12]. The 26−28.5% fat content of WMP is high in polyunsaturated fatty acids which oxidize rapidly during storage [13–16]. Antioxidants are substances added to food to extend shelf life by delaying the onset of oxidation and reducing fat rancidity [17, 18]. Antioxidants scavenge free radicals and inhibit other oxidative reactions [19–21]. Recently, the antioxidant activity of chitosan has received attention with reports finding the antioxidant activity of low molecular weight water soluble chitosan being similar to α−tocopherol [22–24]. Water soluble chitosans will chelate metals in addition to exhibiting a free−radical scavenging effect in the presence of lipids [25]. Incorporation of 0.2%, 0.5% and 1% chitosan of various molecular weights (30, 90 and 120 kDa) into salmon was found to retard lipid oxidation [26]. Low molecular weight chitosan (LMWC), which has high water solubility, may be considered a potential natural antioxidant for stabilizing lipid−containing foods to prolong shelf life [26]. A pH below 6.0 was found to be optimal for achieving desirable antimicrobial and antioxidative−preservative effects with LMWC in liquid and solid foods [27]. Chitosan at low pH (<0.6) inhibited the growth of spoilage organisms in chickpeas and LMWC had higher scavenging activity in apple juice than high

molecular weight chitosan (HMWC) [27]. Furthermore, coating bread with 2% HMWC lowered thiobarbituric acid−reactive substances (TBARS) during storage [28]. Thus, the use of water soluble chitosans with WMP may benefit the dairy industry and other food manufacturers that use WMP as an ingredient by limiting oxidation and extending the shelf life.

RESEARCH OBJECTIVE

The objective of this study was to determine the antioxidant effects of low and high molecular weight water soluble chitosans on the stability of whole milk powder.

MATERIALS AND METHODS

Materials

Whole milk powder was obtained from My Spice Sage Store (Yonkers, New York). Low (LMWC) and high (HMWC) molecular weight−water soluble chitosan powders (9 kDA, degree of deacetylation: 92%, moisture content: 10%, and 90 kDA, degree of deacetylation: 95%, moisture content: NMT 10%, respectively) were obtained from Creative Dynamics INC (BOC SCIENCES is division of Creative Dynamics Inc, Shirley, New York, USA). Chloroform, acetic acid, 2-Thiobarbituric acid (TBA), malonaldehyde bis (1,1,3,3−Tetraethoxypropane),

petroleum ether, potassium iodide (KI), 0.01N sodium thiosulfate solution and other materials were purchased from Sigma−Aldrich, (Saint Louis, Missouri).

Experimental Plan (Figure 3.1)

Concentrations of 2% and 4% LMWC (9 kDa) and 2%, 4% HMWC (90 kDa) solutions were dissolved into 150 g of deionized water (DW) inside a chemical hood by stirring with a sterile spoon for 5 minutes (Figure 3.2). One beaker containing water with no chitosan was used as a control. 150 g of whole milk powder (WMP) was added to each beaker and mixed using a spatula for 15 minutes. The final weights for the beakers containing chitosan solutions with WMP were (303 g, 306 g, 303 g, 306 g in L2, L4, H2, H4, respectively) (L2 = Freeze-dried WMP with 2% LMWC added; L4 = Freeze-dried WMP with 4% LMWC added; H2 = Freeze-dried WMP with 2% HMWC added; H4 = Freeze-dried WMP with 4% HMWC added). All samples were frozen at −80°C for 3 hours to prepare the samples for freeze drying. All samples were freeze dried (LABCONCO, Kansas, Missouri) at −50°C for 94 hours (Figure 3.3). After freeze-drying, the final weight of WMP samples were (151.18 g, 151.38 g, 151.53 g, 151.90 g, 152.53 g in control, L2, L4, H2, H4, respectively). Samples were ground using a mortar and pestle then stored in sealed Whirl-Pak™ bags (Nasco, USA) for 48 days at 45±2°C with RH ranging from 7−19.5% inside an incubator to accelerate oxidation, similar to the procedure used by Osorio [31] (Figure 3.4). Moisture content, water activity, color,

thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) were determined every 8 days for 48 days.

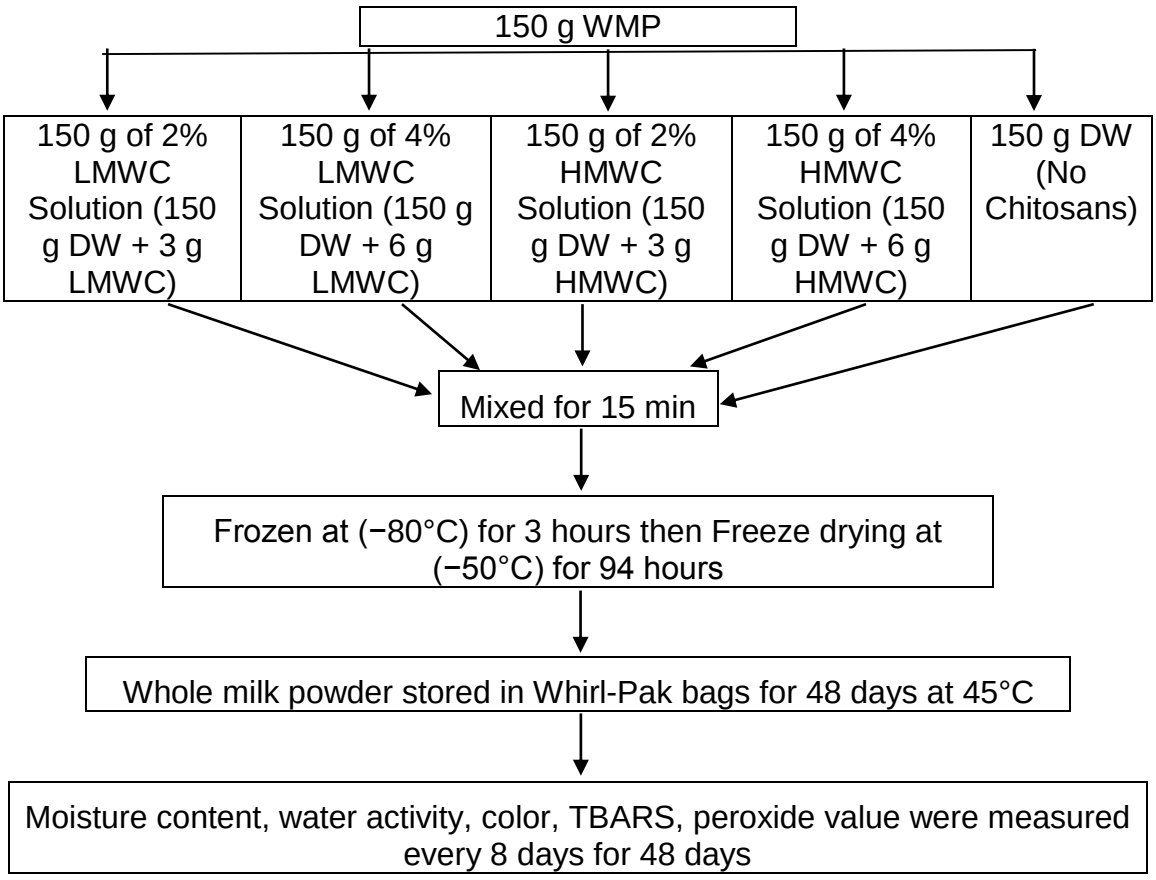

Figure 3.1: Experiment design

Figure 3.2: Low and high molecular weights water soluble chitosan with WMP treatments.

Figure 3.3: WMP inside the freeze dryer

Figure 3.4: WMP after the freeze dryer

Moisture Content

Moisture content was determined using a HB43−S Halogen Moisture Analyzer (Mettler Toledo, Model: HB43−S, Switzerland). Three g of WMP were added to a foil pan then placed in the Halogen Moisture Analyzer. Moisture analyses were run in triplicate for each replicate.

Water Activity

Water activity was determined using an AquaLab *LITE* Water Activity Meter (Decagon Devices, Inc. Pullman, Washington 99163, USA). One g of WMP was added to a sample cup which was placed in Water Activity Meter. Water activity was run in triplicate for each replicate.

Color Determination

Color was determined using a Minolta Colorimeter Model CR-400 (Minolta Corp, Japan). Color was measured through plastic bags and *L, *a, *b, chroma and hue values were recorded. The effect of Whirl-Pack bags on color measurements was compensated for by taking measurements with and without the bag on calibration tiles.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined spectroscopically as described by Lee et al and Raghuveer et al. [29, 30] with modifications. 0.5 g of WMP was placed in a 15 ml Corning™ (VWR North American Cat. No 21008−105, USA) Conical-bottom disposable plastic tube. 4.5 ml deionized water was added to the same tube containing WMP. The tube was mixed for 1 minute using a vortex. Five ml of 20% trichloroacetic acid (TCA) was added and vortex mixed for two minutes. The sample was then centrifuged at 8228 x g for 15 minutes using (Eppendorf 5804R, Germany). The supernatant was filtered using 0.45 µm GHP Membrane Acrodisc (Pall Corporation, USA) and 2 ml of the filtered liquid was placed in another tube with 4 ml TBA solution. The TBA solution was prepared by dissolving 0.2883±0.0005g TBA in hot deionized water (70−75°*C)* then diluted to 100 ml with distilled water. The sample tube was placed in a 75°*C* water bath for 30 minutes. The sample was cooled and the absorbance measured using (Spectronic® 20 GENESYSTEM™, USA) at 535 nm. TBARS values were obtained from a standard curve. A standard curve was prepared using concentrations from 0 µL to 70 µL of a mixture of 1,1,3,3−tetrathoxypropane solution (TEP) and 10% TCA solutions with 4 ml of TBA solution. The tubes used for the standard curve were placed in a 75°*C* water bath and measured the absorbance at 535 nm. The amounts of chitosan in 0.5 gram WMP were 0.01 g, 0.02 g, 0.01 g, and 0.02 g in L2, L4, H2, and H4 treatments respectively. Chitosan affected the absorbance at 535 nm, therefore this was accounted for by measuring the interference and subtracting the milk

sample TBARS value from the chitosan value. TBARS of whole milk powder was determined using this equation: $TBARS_{Milk\ powder} = TBARS_{Milk\ powder\ containing\ chitosan} - TBARS_{Chitosan}$. This equation was applied for each treatment except the control which has no chitosan.

Peroxide Value

The AOAC [31] official methods 28.022 and 50.037 as described by Osorio [32] were used to determine PV with some modifications. Five grams of WMP were weighed using No 1 Whatman filter paper (W&R Balston Limited, England). This sample was wrapped and placed into a silica thimble (22x80 mm). The thimble was then placed into a Pyrex™ sample tube which was inserted into a clip in the Goldfish fat extraction apparatus (Model 35001, Labconco Co., Kansas city, MO, USA). 40 ml of petroleum ether was placed in a 50 ml beaker which was assembled in a retainer ring with a wave washer inserted and twisted to lock. After 8 hours, the petroleum ether was evaporated inside a chemical hood for ~30 min and the amount of fat recovered was determined using this equation: weight of fat = (weight of sample + thimble before extraction) − (dry weight of sample + thimble after extraction). Ten ml of acetic acid/chloroform solvent (3:2) was added to the flask. 0.5 ml of saturated KI solution was added and mixed one minute using a stir bar and then placed inside the chemical hood for two minutes. Fifty ml of distilled water was added, then, 1.0 ml of 1% starch solution was added and the mixture was titrated using 0.01N sodium thiosulfate. The peroxide value was calculated using

the following equation: Peroxide value (meq/kg) = [(0.01 * volume of sodium thiosulfate solution from the titration) / g of fat content] * 1000.

STATISTICAL ANALYSIS

The experiment was replicated five times. All analysis were run in triplicate for each replicate (5 x 3) except for the peroxide value which was run in a single observation for each replicate (5 x 1) because of limited fat content from each extraction. A randomized complete block design with five treatments (control, L2, L4, H2 and H4) was used and ANOVA was used to determine the main effects of treatments and storage days on moisture, water activity, color, TBARS and PV. Statistical analysis was conducted using SAS 9.3 software and means were compared using Tukey test at $\alpha=0.05$.

RESULTS AND DISCUSSION

Moisture content

Moisture content of all treatments significantly decreased during storage ($P<0.05$) (Table 3.1). Moisture content was 2.68% at day 0 for H4 which was significantly different from all other treatments and steadily decreased to a little over 2.20% by day 48 (Table 3.1). At 0, 8, and 24 days, there was no difference in moisture between the control, L2, L4 and H2. H4 samples were significantly lower

in moisture content than other treatments ($P<0.05$) at days 0 and 8. There was no difference between the control and other treatments on days 32, 40, and 48. In general, moisture of control, L2, L4, H2 and H4 samples was lower than original WMP (4.14%) due to freeze-drying (lyophilization) [33]. Labuza [34] found that dried foods become more susceptible to oxidation if their moisture content was less than 2−3%.

Water Activity

Water activity fluctuated during storage for all treatments but remained below 0.12 through 48 days for all treatments (Table 3.2). There was no significant difference in water activity between the control and chitosan treatments at any time ($P>0.05$). Nelson and Labuza [35] stated that between water activity of 0.2 and 0.4, the oxidation rate decreases due to water retarding some initiation and propagation reactions. Nelson and Labuza [35] reported that at very high and very low water activity, lipid oxidation rates are greater than for intermediate levels. In this experiment, water activity ranged from 0.004 to 0.120 which was extremely low and in an optimal for lipid oxidation.

Color

Appearance is an important characteristic of foods as this is the first impression a consumer has to evaluate food quality. The L* value scale is from 0

(black) to 100 (white). The L* value for chitosan alone was 79.99 (LMWC) and 72.89 (HMWC) while the L* value for WMP alone was 95.14. Thus, the addition of chitosan to the WMP generally reduced the L* value of the mixture (Table 3.3). In day 0, there was no significant difference in lightness between control and L2, L4, H2 samples, but H4 was significantly darker than other treatments (control, L2, L4, H2) ($P<0.05$). L2 samples were significantly darker than control ($P<0.05$) from day 32 until day 48. L4, H2 and H4 samples were significantly darker than control ($P<0.05$) from day 8 until day 48. L4 samples were significantly darker than L2 on days 8, 16, 24 and 32 ($P<0.05$) but L4 and L2 samples were not significantly different on days 40 and 48. Based on L* values, increasing the concentrations of chitosan, generally resulted in a darker appearance of WMP.

The a* value represents redness/greenness of a sample ranging from 60 (red) to −60 (green). All treatments were in the negative (green) range (Table 3.4). Control, L2 and L4 samples had significantly lower a* values than H2 and H4 ($P<0.05$). The a* value for chitosan alone was 0.55 (LMWC) and 4.79 (HMWC) with the a* value for WMP alone being −3.11. The addition of chitosan to the WMP generally increased the a* value of the mixture. There was no significant difference in a* value between L4 and control samples at days 8, 16, 24, 32, 40. L2 samples had lower a* value than L4 at days 0 and 40 ($P<0.05$) but L4 and L2 samples did not differ on 8, 16, 24, 32, 48 days. As expected, due to the higher a* of HMWC. H4 had higher a* values than H2 (and all other treatments at all sampling days) ($P<0.05$). Generally, increasing concentrations of HMWC (H2 and H4), increased

a* value ($P<0.05$). The a* values of control and L2 samples significantly increased during storage ($P<0.05$) while H2 and H4 were stable during storage ($P<0.05$). For L4, the a* values decreased up to day 24 and then increased up to 40 and then decreased up to day 48.

The b* values represents yellowness/blueness of a sample ranging from 60 (yellow) to −60 (blue). WMP b* values were all in the positive b* range (Table 3.5). Interestingly, the b* for LMWC alone was below (13.45) that of WMP alone (15.28) while the b* value of HMWC was above (22.09) that of WMP. The change in b* value when the chitosan alone mixed with WMP reflected this difference in b* by lowering (LMWC) or raising (HMWC) the final b* value of the mixture. There was no significant difference between control and L2 samples during storage except on day 48. H2 and H4 samples had higher b* values compared to controls except on days 0 and 16 ($P<0.05$). Increasing the concentration of low and high molecular weight chitosan (L2, L4, H2 and H4 samples) did not significantly affect the b* value of WMP samples ($P<0.05$). The b* value of chitosan treatments was higher than control during storage. Nielsen et al. [36] indicated that the b-value was correlated with non-enzymatic browning in whey powder and its increase in milk powder is related to Maillard reactions that increased at high water activity and high temperature. However, the water activity of WMP samples during this experiment remained between 0.004 to 0.120. WMP with chitosan had higher b* values which increased during storage (controls did not) and had a longer oxidative shelf life [36] both of wich may be due to Maillard reaction products [36].

Chroma (C*) describes color saturation, numerically quantified as [$C^* = (a^{*2} + b^{*2})^{1/2}$ in CIE Lab color space is a measure of color intensity. The C* value for LMWC (13.46) alone was below that of WMP alone (15.60) while the C* value of HMWC (22.53) was above that of WMP. The change in C* value when chitosan was mixed with WMP reflected the differences in chroma by lowering (LMWC) on raising (HMWC) the final C* value of the mixture. There was no significant difference between control and L2 samples during storage except on days 32 and 40 (Table 3.6). There was no significant difference between control and L4 samples during storage except on days 0 and 32. H2 samples had higher C* values compared to controls on days 8, 24, 32, 40 and 48 ($P<0.05$). H4 samples had higher C* values compared to controls on days 32, 40, and 48 ($P<0.05$).

Hue angle (H) reflects the true color of a material ranging through the red-yellow-green-blue wavelengths of the visible spectrum, numerically quantified as [$hue = (\tan^{-1} b^*/a^*)$. A hue angle of 0/360° is red, 90° is yellow, 180° is green and 270° is blue [37]. The hue for all treatments significantly decreased during storage ($P<0.05$) (Table 3.7). Control, L2 and L4 samples had significantly higher H values than H2 and H4 ($P<0.05$). The hue for chitosan alone was 87.67 (LMWC) and 78.78 (HMWC) with the hue for WMP alone being 101.50. The addition of chitosan to the WMP generally increased the hue angle of the mixture. There was no significant difference in hue between control and L2 samples during storage except on days 0, 16 and 48. L4 samples had lower hue angle than control during storage except at day 24 ($P<0.05$) but L4 and L2 samples did not differ during storage. H4

samples had lower hue angle than H2 ($P<0.05$). Generally, increasing concentrations of HMWC (H2 and H4), decreased hue angle ($P<0.05$).

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS of all treatments significantly increased during storage ($P<0.05$) (Table 3.8). There was no significant difference between control and L2 throughout storage ($P<0.05$) (Table 3.8). L4 samples had significantly lower TBARS than control on days 0, 16, 24, 40, and 48 ($P<0.05$). TBARS of H2 and H4 treatments had lower TBARS than control on days 0 and 16 ($P<0.05$) but there was no significant difference between H2, H4 and control on 8, 24 and 32 days of storage. H2 and H4 treatments had significantly higher TBARS than control on days 40 and 48 ($P<0.05$). H2 samples were not different from H4 during storage. L4 had significantly lower TBARS than L2 in 0, 16, 40 and 48 days ($P<0.05$). The 4% LMWC was generally better than other treatments in preventing TBARS increase possibly due to the high scavenging activity of chitosan against hydroxyl radicals (·OH) which is related to the amino group in the chitosan structure [38]. Moreover, the scavenging rate increased with concentration [38]. LMWC was theorized to exhibit a stronger antioxidative effect than HMWC due to the reduced exposure of amine groups held within the more compact structure of HMWC resulting from the greater number of intramolecular hydrogen bonds in HMWC compared to LMWC [26]. Xing et al. [40] reported that LMWC (9 kDA) had a stronger scavenging effect on ($O_2^{\cdot -}$) and (·OH) than HMWC and that the scavenging rate and reducing power

of chitosan increased with increasing concentration. Matsugo et al. [41] reported that water soluble chitosan inhibited TBARS formation via the radical chain breaking activity through the contribution of aminoglycosides. Maillard reactions also occur between the amino groups in chitosan and reducing sugars such as lactose present in WMP producing antioxidants during storage especially with relatively high temperatures and long storage times. Phisut and Jiraporn [39] reported that Maillard reaction products derived from chitosan-lactose had greater scavenging capacity reducing powder and inhibition of lipid peroxidation than chitosan alone. A higher concentration of LMWC increased the number of total amine groups available for the Maillard reactions [26].

Kamil et al. [42] reported that molecular weight determines metal ion chelation ability because cationic amino groups of chitosan conveys intramolecular electrostatic repulsive forces, which increase the hydrodynamic volume in LMWC by extended chain conformation in their charged state. This may account for the relatively lower chelation by HMWC [42].

Peroxide Value

PV test measures peroxide formation during the first stages of oxidation. All treatments started with low PV which increased during storage ($P<0.05$) (Table 3.9). For treatments L2, H2 and H4 there were lower PV than controls on days 16, 24, 32 ($P<0.05$). L4 treatment had significantly lower peroxide values than control

samples at 16, 24, 32, 40 and 48 days ($P<0.05$) possible due to the stronger scavenging activity and metals chelating of LMWC. Furthermore, the scavenging activity increased with higher levels of LMWC. Mao and Wu [43] found that PV of samples with chitosan were lower than control. Kamil et al. [42] found that LMWC was more effective than the HMWC in preventing lipid oxidation.

In this experiment, WMP was stored at 45°C which decreases the shelf life because autoxidation increase at high temperature. WMP is commonly stored between 20−25°C and based on the general Q_{10} rule for chemical reactions, the rate is doubled for each temperature increase of 10°C. Thus, the retardation of oxidation observed of WMP with 4% LMWC observed in the relatively high storage temperature used in the current study would have a greater shelf life extension effect with WMP stored at lower temperatures.

Table 3.1: Effect of low and high molecular weights water soluble chitosan on the moisture content of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	3.05 ± 0.20^{av}	3.04 ± 0.40^{av}	3.00 ± 0.30^{av}	3.12 ± 0.30^{av}	2.68 ± 0.05^{bv}
8	2.63 ± 0.02^{aw}	2.66 ± 0.24^{aw}	2.68 ± 0.20^{aw}	2.62 ± 0.20^{aw}	2.34 ± 0.20^{bw}
16	2.60 ± 0.02^{aw}	2.36 ± 0.20^{bx}	2.50 ± 0.20^{abx}	2.50 ± 0.20^{abx}	2.62 ± 0.10^{av}
24	2.22 ± 0.10^{cx}	2.39 ± 0.10^{acx}	2.50 ± 0.10^{abx}	2.40 ± 0.01^{bcxy}	2.46 ± 0.04^{bx}
32	2.25 ± 0.10^{ax}	2.30 ± 0.20^{ax}	2.24 ± 0.10^{ay}	2.30 ± 0.20^{ay}	2.45 ± 0.10^{awx}
40	2.26 ± 0.10^{ax}	2.13 ± 0.02^{ay}	1.94 ± 0.02^{abz}	2.06 ± 0.04^{az}	2.11 ± 0.10^{ay}

Values are presented as mean ± standard deviation.

$^{a-c}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{v-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.2: Effect of low and high molecular weights water soluble chitosan on the water activity of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	0.032 ± 0.01^{az}	0.006 ± 0.002^{az}	0.004 ± 0.001^{az}	0.014 ± 0.003^{ay}	0.013 ± 0.003^{ay}
8	0.079 ± 0.03^{ay}	0.074 ± 0.01^{ay}	0.084 ± 0.02^{ax}	0.097 ± 0.03^{ax}	0.069 ± 0.01^{ax}
16	0.120 ± 0.06^{ax}	0.097 ± 0.04^{axy}	0.096 ± 0.04^{ax}	0.095 ± 0.03^{ax}	0.092 ± 0.05^{ax}
24	0.098 ± 0.04^{axy}	0.105 ± 0.05^{axy}	0.104 ± 0.05^{ax}	0.097 ± 0.04^{ax}	0.097 ± 0.01^{ax}
32	0.029 ± 0.01^{az}	0.034 ± 0.01^{az}	0.035 ± 0.002^{ay}	0.038 ± 0.01^{ay}	0.030 ± 0.01^{ay}
40	0.114 ± 0.05^{ax}	0.110 ± 0.05^{ax}	0.106 ± 0.04^{ax}	0.090 ± 0.03^{ax}	0.087 ± 0.04^{ax}
48	0.033 ± 0.002^{az}	0.033 ± 0.001^{az}	0.031 ± 0.003^{ayz}	0.033 ± 0.002^{ay}	0.033 ± 0.001^{ay}

Values are presented as mean ± standard deviation.

$^{a-b}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{x-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.3: Effect of low and high molecular weights water soluble chitosan on the lightness (L* value) of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	95.14 ± 0.08^{ax}	95.09 ± 0.34^{axy}	94.88 ± 0.10^{abw}	94.65 ± 0.27^{abwy}	94.26 ± 0.27^{bwx}
8	96.32 ± 0.27^{aw}	95.73 ± 0.14^{abw}	94.44 ± 0.41^{cdwx}	95.04 ± 0.08^{cbwx}	94.11± 0.09^{dwy}
16	95.88 ± 0.45^{aw}	95.26 ± 0.23^{abwy}	94.54 ± 0.34^{cwx}	94.66 ± 0.33^{cbwy}	93.99± 0.47^{cwxz}
24	96.08 ± 0.47^{aw}	95.51 ± 0.13^{awx}	94.48 ± 0.48^{bwx}	94.51 ± 0.27^{by}	94.18± 0.31^{bwx}
32	96.34 ± 0.39^{aw}	95.41 ± 0.14^{bwx}	94.43 ± 0.20^{cwx}	94.62 ± 0.20^{dxy}	94.15± 0.36^{cdxy}
40	96.32 ± 0.37^{aw}	94.89 ± 0.49^{bcy}	94.50 ± 0.26^{cdwx}	94.41 ± 0.50^{cdyz}	93.82± 0.48^{dxyz}
48	96.05 ± 0.42^{aw}	94.87 ± 0.35^{by}	94.27 ± 0.39^{bcx}	93.97 ± 0.37^{cz}	93.58± 0.32^{cz}

Values are presented as mean ± standard deviation.

$^{a-d}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{w-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.4: Effect of low and high molecular weights water soluble chitosan on the greenness (−a* value) of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	-3.11 ± 0.06^{ez}	-2.69 ± 0.06^{dx}	-2.50 ± 0.07^{cwx}	-2.07 ± 0.03^{bw}	-1.72 ± 0.03^{awx}
8	-2.60 ± 0.03^{dxy}	-2.58 ± 0.05^{cdw}	-2.49 ± 0.06^{cdw}	-2.07 ± 0.03^{bw}	-1.67 ± 0.03^{aw}
16	-2.65 ± 0.08^{dy}	-2.58 ± 0.07^{cdw}	-2.50 ± 0.12^{cwx}	-2.09 ± 0.04^{bw}	-1.72 ± 0.05^{awx}
24	-2.55 ± 0.14^{cx}	-2.61 ± 0.03^{cwx}	-2.58 ± 0.04^{cxy}	-2.05 ± 0.03^{bw}	-1.70 ± 0.04^{awx}
32	-2.43 ± 0.06^{cw}	-2.52 ± 0.11^{cw}	-2.48 ± 0.04^{cw}	-2.04 ± 0.04^{bw}	-1.66 ± 0.02^{aw}
40	-2.44 ± 0.02^{dw}	-2.55 ± 0.06^{cw}	-2.43 ± 0.05^{dw}	-2.05 ± 0.02^{bw}	-1.65 ± 0.04^{aw}
48	-2.44 ± 0.04^{cw}	-2.53 ± 0.05^{cdw}	-2.62 ± 0.07^{dy}	-2.11 ± 0.05^{bw}	-1.78 ± 0.02^{ax}

Values are presented as mean ± standard deviation.

$^{a-e}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{w-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.5: Effect of low and high molecular weights water soluble chitosan on the yellow color (+b* value) of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	15.28 ± 0.26^{av}	14.53 ± 0.15^{abx}	13.92 ± 0.15^{bz}	15.31 ± 0.13^{ayz}	15.28 ± 0.16^{axy}
8	14.54 ± 0.04^{bwy}	14.78 ± 0.11^{abwx}	14.55 ± 0.33^{bxy}	15.68 ± 0.10^{axy}	15.20 ± 0.02^{aby}
16	15.17 ± 1.00^{avx}	14.96 ± 0.22^{awx}	14.92 ± 0.52^{awx}	15.96 ± 0.25^{awx}	15.84 ± 0.66^{aw}
24	15.04 ± 1.22^{bvw}	15.29 ± 0.14^{abvw}	15.23 ± 0.21^{abw}	16.16 ± 0.17^{awx}	15.77 ± 0.43^{abwx}
32	14.22 ± 0.26^{by}	15.27 ± 0.22^{abvw}	15.26 ± 0.33^{abw}	16.23 ± 0.22^{aw}	15.95 ± 0.58^{aw}
40	14.26 ± 0.18^{cy}	15.44 ± 0.30^{abv}	15.18 ± 0.20^{bcw}	16.42 ± 0.23^{aw}	16.07 ± 0.65^{abw}
48	14.69 ± 0.22^{cwxy}	15.77 ± 0.25^{bv}	16.03 ± 0.31^{abv}	17.05 ± 0.25^{av}	17.03 ± 0.25^{av}

Values are presented as mean ± standard deviation.

$^{a-c}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{v-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.6: Effect of low and high molecular weights water soluble chitosan on the chroma (C* value) of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	15.60 ± 0.25^{av}	14.78 ± 0.16^{abx}	14.14 ± 0.16^{bz}	15.45 ± 0.12^{ayz}	15.38 ± 0.16^{axy}
8	14.77 ± 0.04^{bwx}	15.00 ± 0.10^{abwx}	14.77 ± 0.34^{bwy}	15.82 ± 0.10^{axy}	15.29 ± 0.04^{abx}
16	15.20 ± 0.77^{avw}	15.18 ± 0.22^{avwx}	15.13 ± 0.53^{awxy}	16.09 ± 0.25^{awx}	15.93 ± 0.66^{awy}
24	15.25 ± 1.23^{bvw}	15.51 ± 0.14^{abvw}	15.45 ± 0.23^{abx}	16.29 ± 0.17^{awx}	15.86 ± 0.43^{abwx}
32	14.42 ± 0.27^{bx}	15.48 ± 0.23^{avw}	15.47 ± 0.33^{ax}	16.35 ± 0.22^{awx}	16.04 ± 0.58^{aw}
40	14.47 ± 0.18^{cx}	15.65 ± 0.31^{abv}	15.38 ± 0.21^{bcwx}	16.59 ± 0.20^{awv}	16.00 ± 0.95^{abw}
48	15.26 ± 0.79^{cvw}	15.71 ± 0.58^{cv}	16.20 ± 0.31^{cbv}	17.07 ± 0.41^{abv}	17.12 ± 0.25^{abv}

Values are presented as mean ± standard deviation.

$^{a-c}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{v-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.7: Effect of low and high molecular weights water soluble chitosan on the hue angle (H value) of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	101.50 ± 0.13^{au}	100.49 ± 0.15^{bu}	100.19 ± 0.19^{bcu}	97.68 ± 0.14^{duv}	96.42 ± 0.06^{euv}
8	100.14 ± 0.13^{av}	99.90 ± 0.26^{abvw}	99.72 ± 0.04^{bvw}	97.50 ± 0.06^{cvwx}	96.27 ± 0.09^{dvw}
16	100.84 ± 0.63^{aw}	99.79 ± 0.20^{bvw}	99.52 ± 0.13^{bcvwx}	97.45 ± 0.15^{dvw}	96.19 ± 0.10^{evwx}
24	99.62 ± 0.27^{ax}	99.67 ± 0.10^{abwxy}	99.61 ± 0.07^{abwx}	97.23 ± 0.16^{cwxy}	96.14 ± 0.26^{dvwx}
32	99.68 ± 0.14^{ax}	99.38 ± 0.26^{abyz}	99.24 ± 0.15^{bxy}	97.18 ± 0.08^{cwxy}	95.95 ± 0.25^{dwx}
40	99.69 ± 0.06^{ax}	99.37 ± 0.08^{abyz}	99.06 ± 0.06^{by}	97.16 ± 0.09^{cwxy}	95.88 ± 0.12^{dx}
48	99.75 ± 0.77^{ax}	99.14 ± 0.14^{bz}	99.24 ± 0.11^{bxy}	96.94 ± 0.50^{cy}	95.98 ± 0.06^{dwx}

Values are presented as mean ± standard deviation.

$^{a-e}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{u-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Table 3.8: Effect of low and high molecular weights water soluble chitosan on the TBARS values of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	0.081 ± 0.017^{ax}	0.069 ± 0.025^{abx}	0.048 ± 0.021^{cx}	0.060 ± 0.008^{cby}	0.042 ± 0.016^{cz}
8	0.070 ± 0.014^{ax}	0.067 ± 0.008^{ax}	0.052 ± 0.017^{ax}	0.065 ± 0.002^{ay}	0.065 ± 0.002^{ay}
16	0.119 ± 0.011^{aw}	0.105 ± 0.019^{abw}	0.079 ± 0.019^{cw}	0.087 ± 0.010^{cbx}	0.090 ± 0.008^{bcx}
24	0.128 ± 0.024^{avw}	0.122 ± 0.034^{abvw}	0.105 ± 0.037^{bv}	0.114 ± 0.033^{abw}	0.114 ± 0.034^{abw}
32	0.143 ± 0.016^{auv}	0.138 ± 0.022^{auv}	0.126 ± 0.027^{au}	0.143 ± 0.016^{av}	0.143 ± 0.016^{av}
40	0.163 ± 0.022^{bt}	0.154 ± 0.025^{btu}	0.125 ± 0.021^{cuv}	0.184 ± 0.023^{au}	0.190 ± 0.026^{au}
48	0.180 ± 0.002^{bt}	0.168 ± 0.016^{bt}	0.146 ± 0.010^{ct}	0.210 ± 0.003^{at}	0.210 ± 0.003^{at}

Values are presented as mean ± standard deviation.

$^{a-c}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{t-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

TBARS values are expressed as mg malonaldehyde/kg WMP.

Table 3.9: Effect of low and high molecular weights water soluble chitosan on the peroxide values of whole milk powder

Days	Control	L 2	L 4	H 2	H 4
0	8.64 ± 2.12^{ax}	7.04 ± 1.90^{ay}	6.72 ± 2.27^{ay}	7.56 ± 1.84^{az}	7.08 ± 1.95^{ay}
8	9.24 ± 0.50^{ax}	8.37 ± 0.08^{axy}	8.11 ± 0.12^{axy}	8.41 ± 0.72^{ayz}	9.21 ± 0.75^{axy}
16	14.72 ± 2.72^{aw}	10.59 ± 2.23^{bwx}	9.44 ± 3.36^{bx}	10.47 ± 2.13^{bxy}	10.81 ± 1.58^{bwx}
24	16.69 ± 0.79^{avw}	12.60 ± 1.70^{bvw}	12.40 ± 1.28^{bvw}	13.53 ± 1.80^{bw}	13.29 ± 1.76^{bvw}
32	14.85 ± 0.34^{aw}	11.03 ± 2.01^{bw}	10.11 ± 1.53^{bwx}	11.72 ± 1.84^{bwx}	11.62 ± 1.87^{bvwx}
40	16.08 ± 0.27^{auw}	13.87 ± 0.50^{abv}	13.06 ± 0.37^{bv}	14.17 ± 0.81^{abvw}	13.83 ± 0.49^{abv}
48	18.64 ± 0.90^{auv}	16.90 ± 1.05^{abu}	15.68 ± 0.90^{bu}	17.07 ± 0.89^{abu}	17.07 ± 1.14^{abu}

Values are presented as mean ± standard deviation.

$^{a-b}$ means within rows with different superscript are significantly different ($P < 0.05$).

$^{u-z}$ means within columns with different superscript are significantly different ($P < 0.05$).

Low molecular weight = 9 kDa; High molecular weight = 90 kDa; L2 = Freeze-dried WMP with 2% low molecular weight chitosan added; L4 = Freeze-dried WMP with 4% low molecular weight chitosan added; H2 = Freeze-dried WMP with 2% high molecular weight chitosan added; H4 = Freeze-dried WMP with 4% high molecular weight chitosan added.

Peroxide values are expressed as milliequivalents of peroxide per kg WMP fat.

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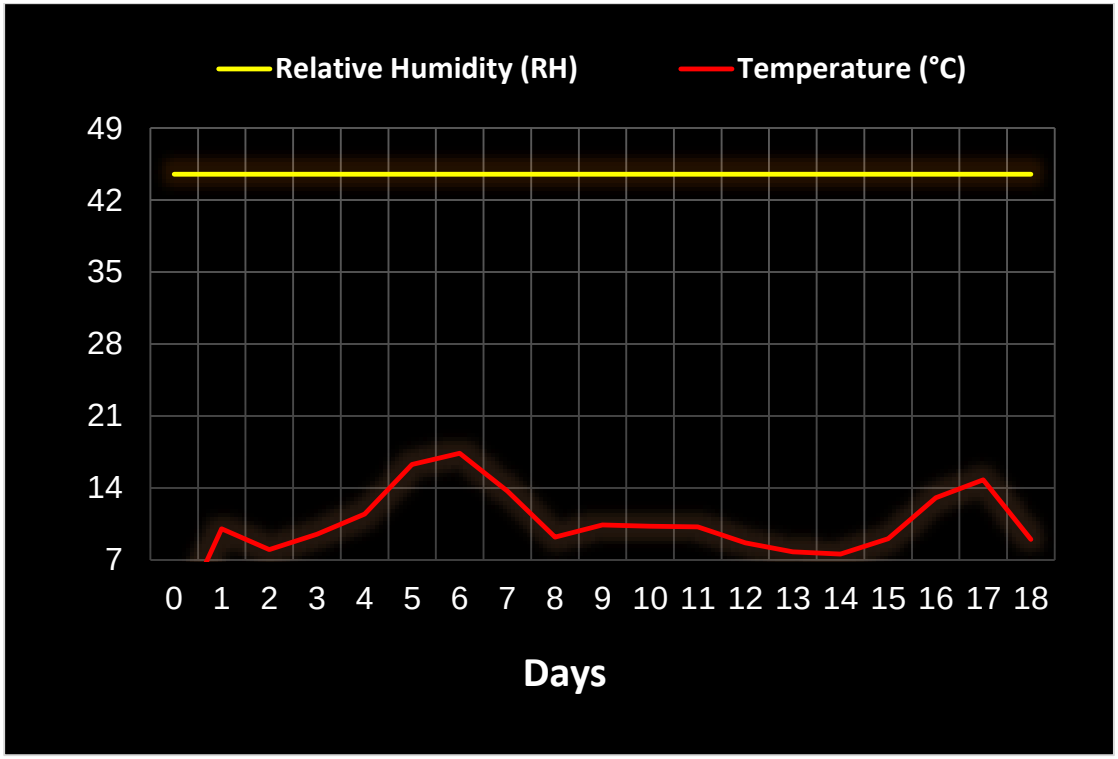
CONCLUSION

TBARS and PV results indicated that LMWC (L4) was significantly slowed lipid oxidation (PV and TBARS) compared to other treatments tested. However, the added cost of LMWC (~$2.40 per 40g) would need to be balanced against the extension of shelf and possible savings in reduction of losses due to oxidation. The cost of 1 kg WMP without chitosan is ~$15, thus, 1 kg WMP with 4% chitosan would cost ~$17.40.

APPENDICES

Appendix A



Relative humidity and temperature (45±2°C) during storage

Appendix B

TBARS Test

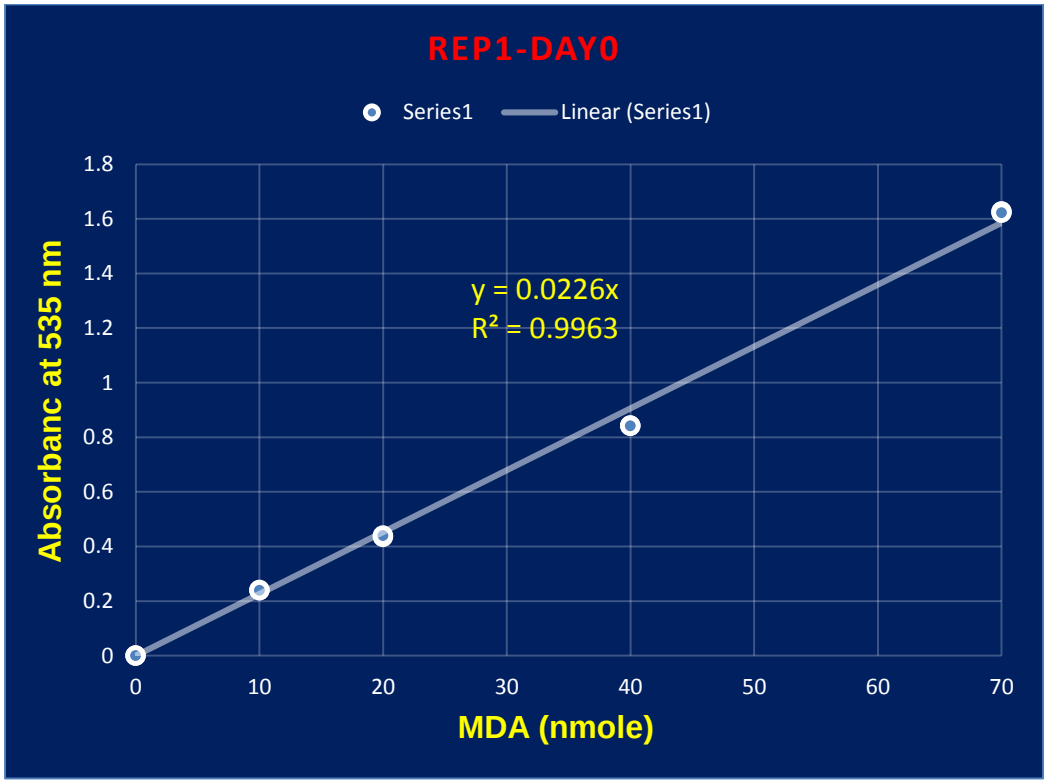
Standard curve:

1- First, we made 3 fresh solutions every test and placed them in a dark place:
   a. TCA 10% Solution: 10 grams of Trichloroacetic acid (TCA) was added to 100 ml volumetric flask then diluted to 100 ml with distilled water.
   b. 1,1,3,3−Tetrathoxypropane (TEP) Solution: 25 g TCA + 61.800 µL Malonaldehyde bis [1,1,3,3−Tetrathoxypropane] was added to 250 ml volumetric flask then diluted to 250 ml with distilled water.
   c. TBA Solution: 0.2883g of 2−Thiobarbituric acid added to 50 ml beaker with some hot water to break the particles using spatula then transferred the solution to 100 ml volumetric flask then diluted to 100 ml with distilled water.
2- Five tubes were duplicated to 10 tubes. All these tubes were full by:
   a. 0 µL of TEP solution + 2 ml 10%TCA solution.
   b. 10 µL of TEP solution + 1.99 ml 10%TCA solution.
   c. 20 µL of TEP solution + 1.98 ml 10%TCA solution.
   d. 40 µL of TEP solution + 1.96 ml 10%TCA solution.
   e. 70 µL of TEP solution + 1.93 ml 10%TCA solution.
3- A 4 ml of TBA solution was added to the ten tubes.
4- The tubes were placed in water bath at 75°C for 30 minutes.

5- The absorbance was read at 535 nm using spectrophotomenter model 20 Genesys™ (USA).

An example of standard curve using Microsoft Excel: DAY 0 Test

MDA	Abs (535 nm)
0	0
10	0.24
20	0.435
40	0.843
70	1.627
0	0
10	0.239
20	0.439
40	0.841
70	1.622



Standard curve

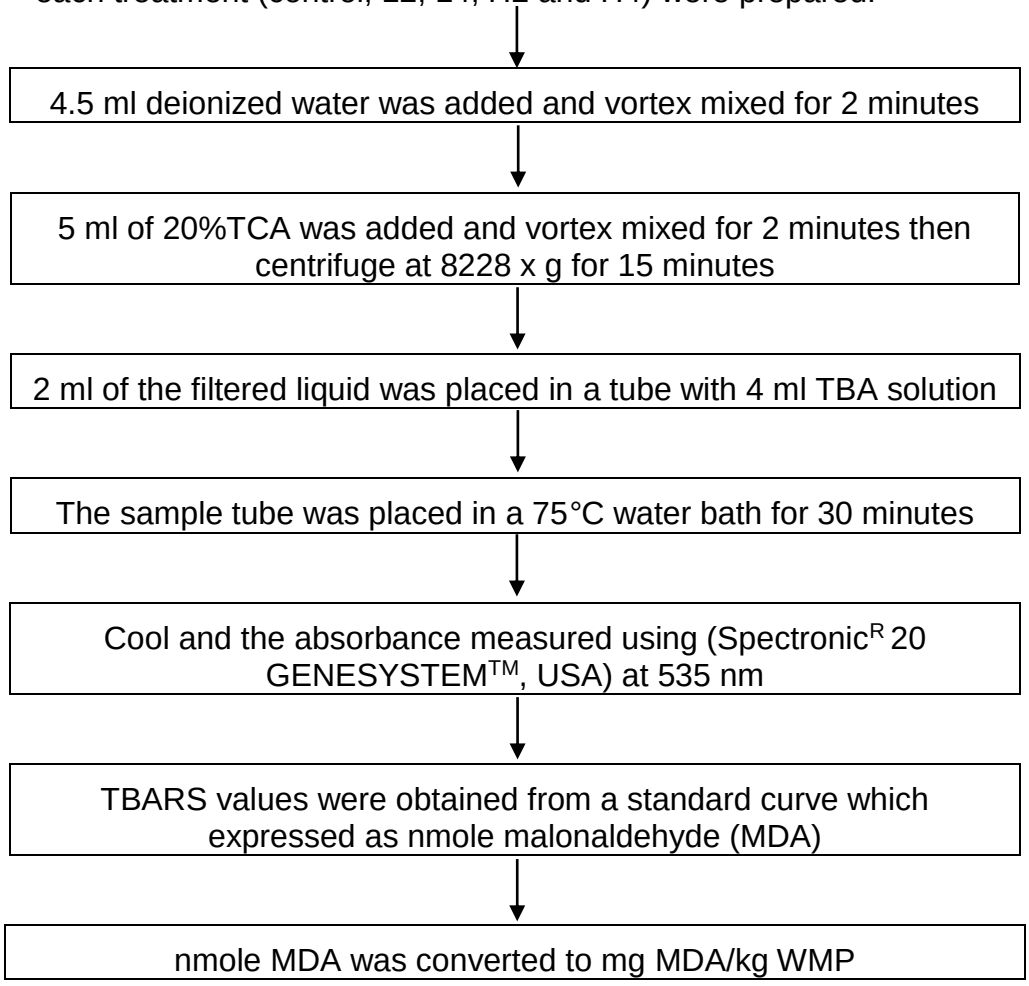
TBARS Test: TBARS of Milk Powder Containing Chitosan

1- First, we made 3 fresh solutions every test and placed them in a dark place:

a. TCA 20% Solution: 20 grams of Trichloroacetic acid (TCA) was added to 100 ml volumetric flask then diluted to 100 ml with distilled water.

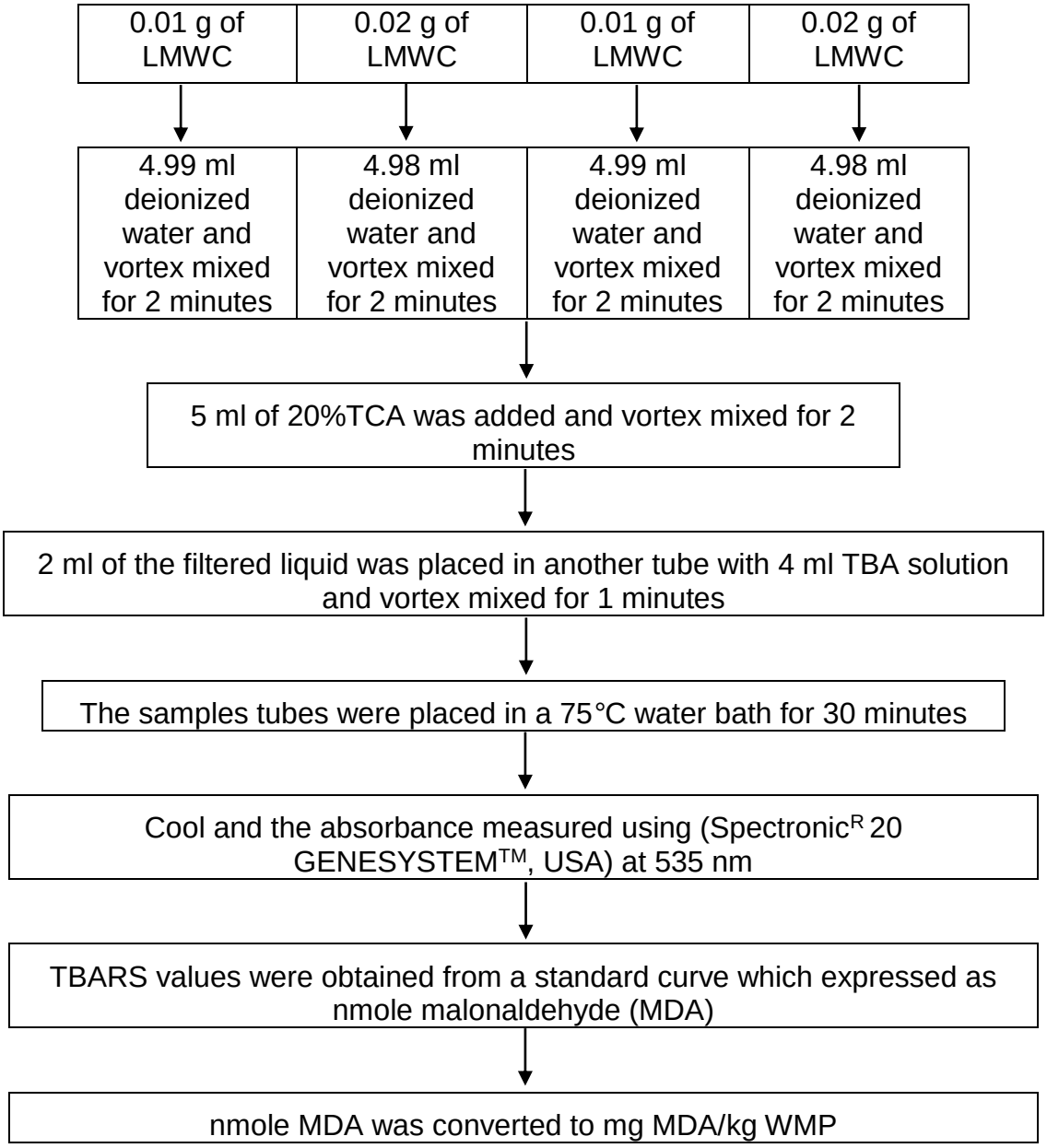
b. TBA Solution: 0.2883g of 2-Thiobarbituric acid added to 50 ml beaker with some hot water to break the particles using spatula then transferred the solution to 100 ml volumetric flask then diluted to 100 ml with distilled water.

2- Five of 15 mL conical bottom disposable plastic tubes contain 0.5 g from each treatment (control, L2, L4, H2 and H4) were prepared.

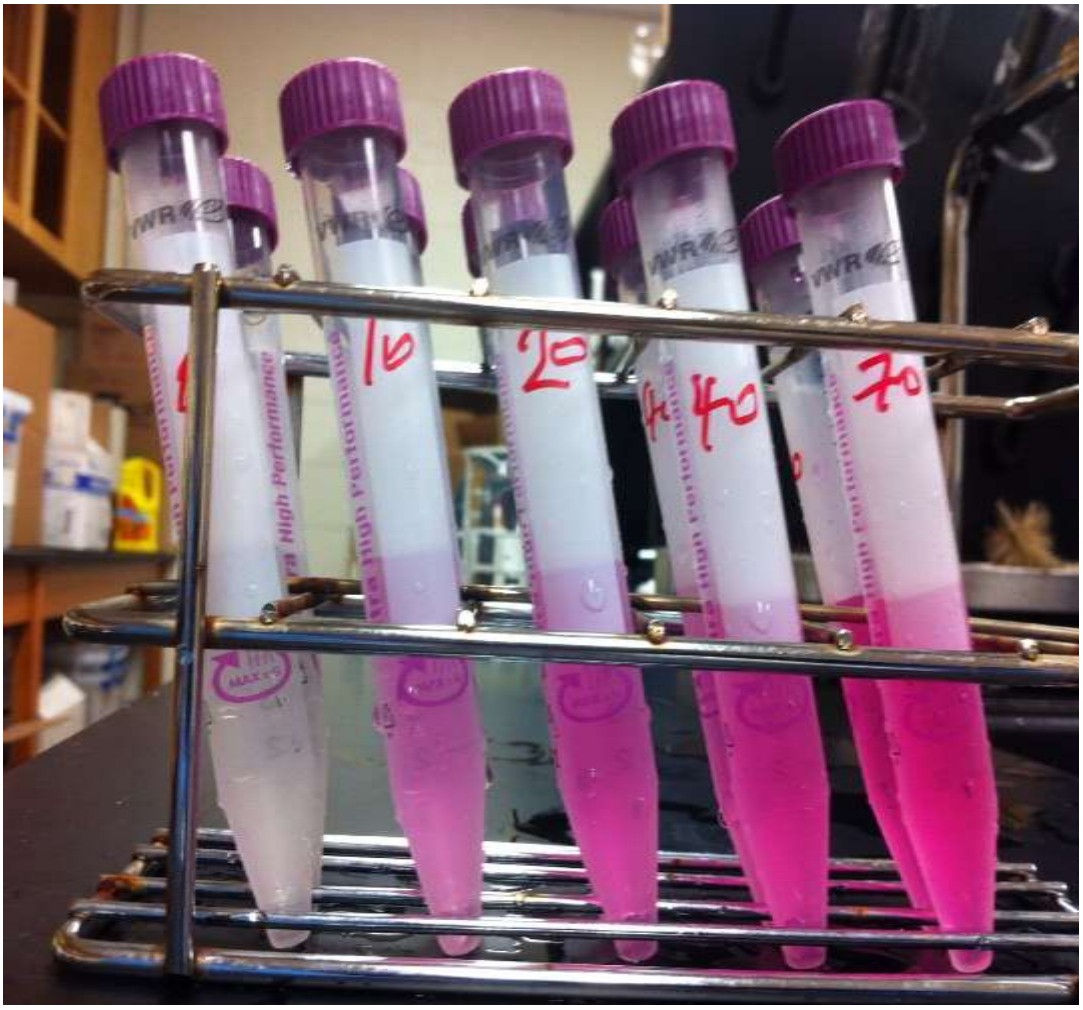


TBARS Test: TBARS of Chitosan Alone

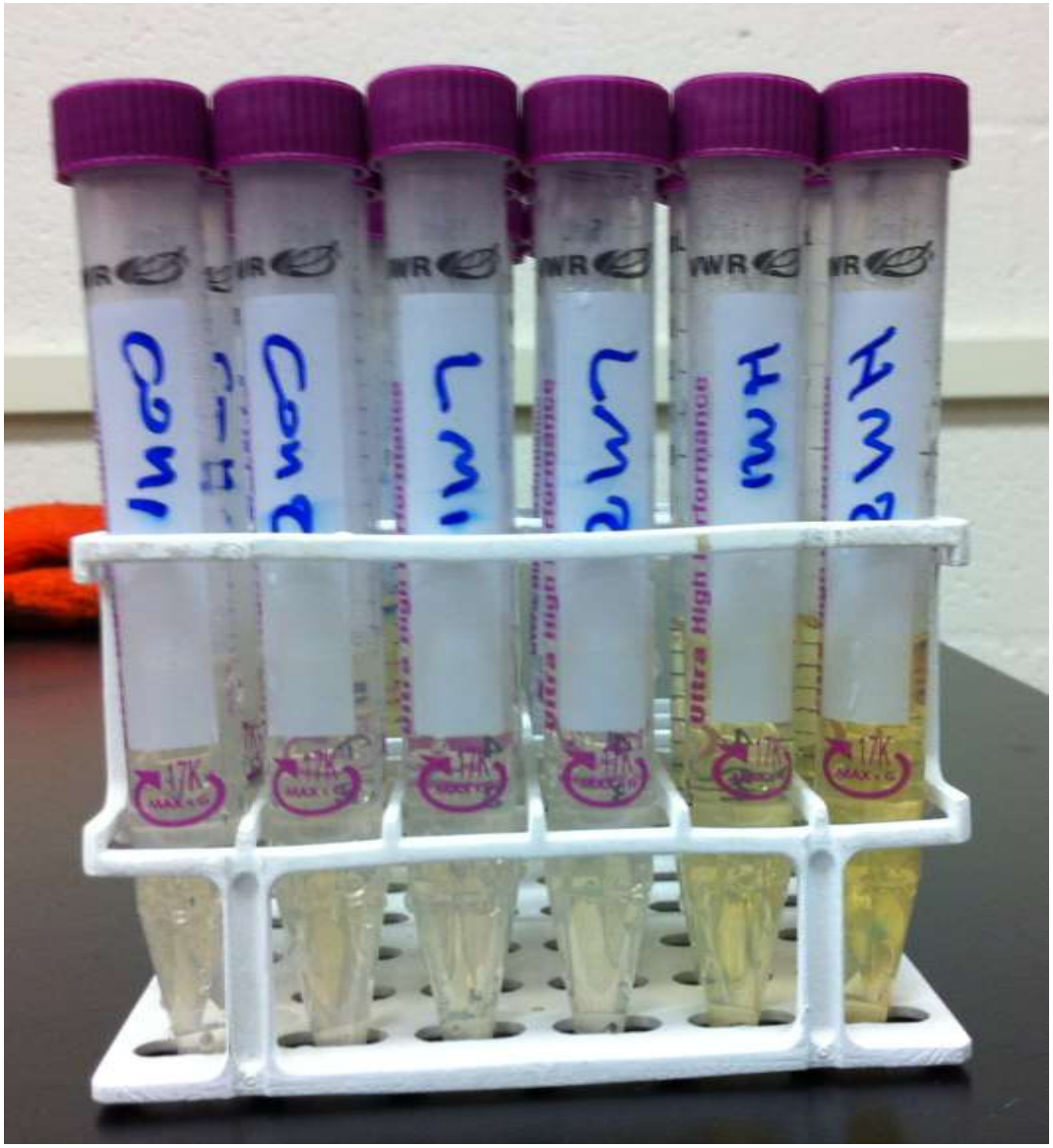
TBARS of chitosan amounts in 0.5 g WMP samples were determined as the following:



TBARS Milk powder = **TBARS** Milk powder containing chitosan – **TBARS** Chitosan



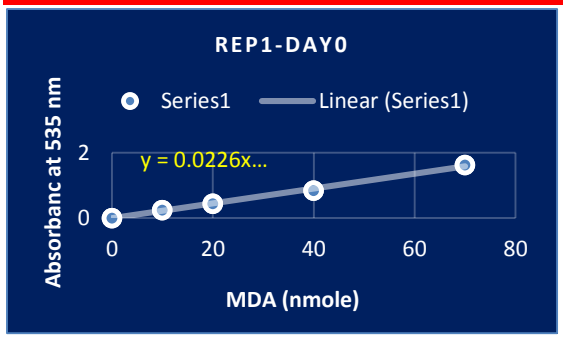
TBARS test of a standard curve

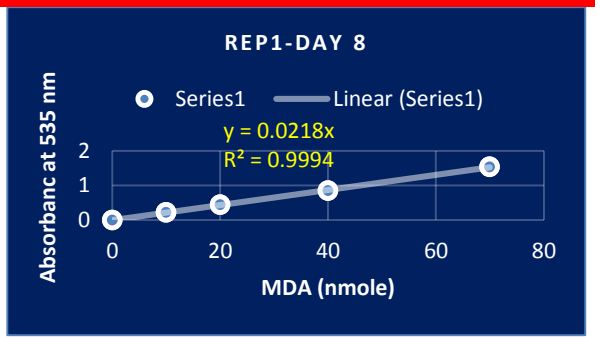


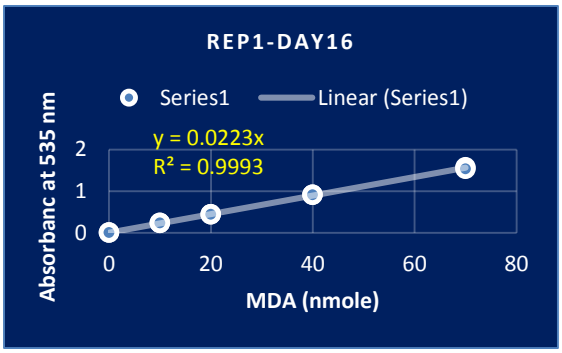
TBARS test of whole milk powder samples (WMP)

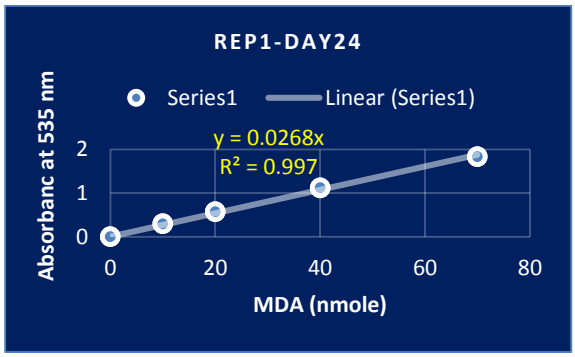
Appendix C

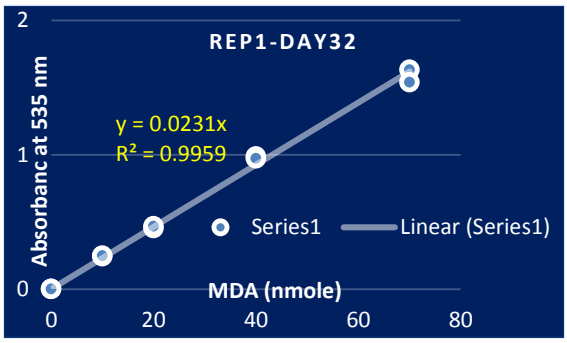
TBARS Standard Curves – First Replication

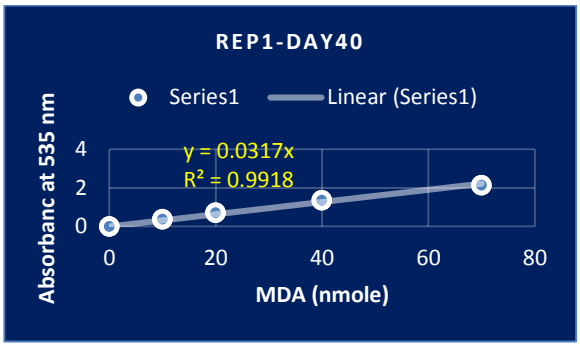


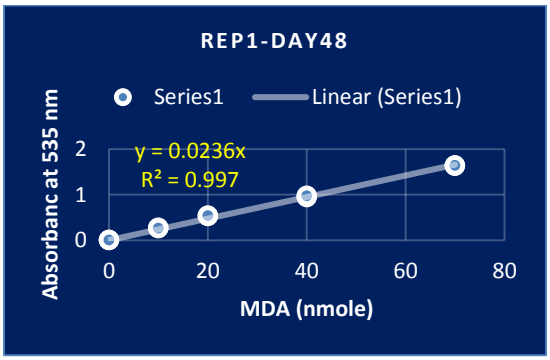




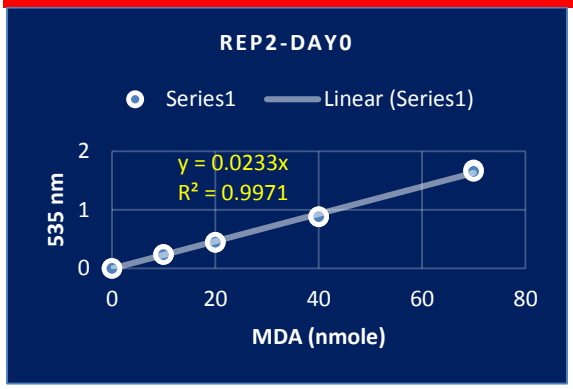


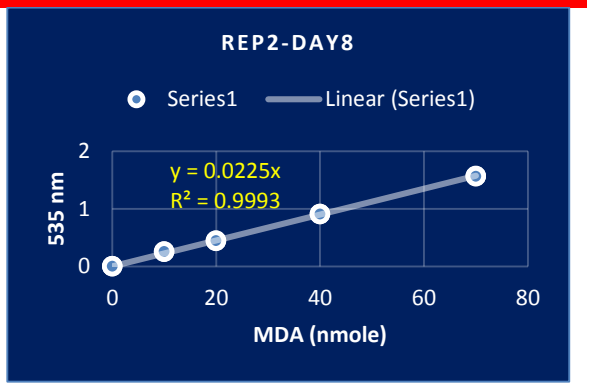


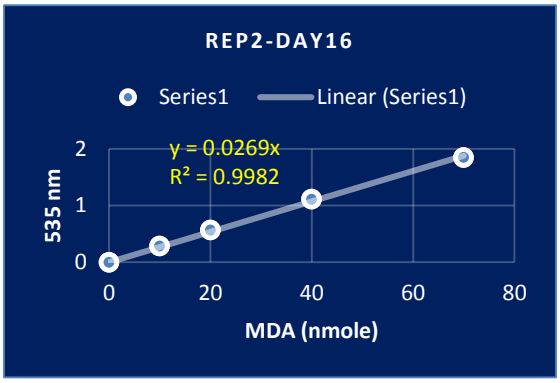


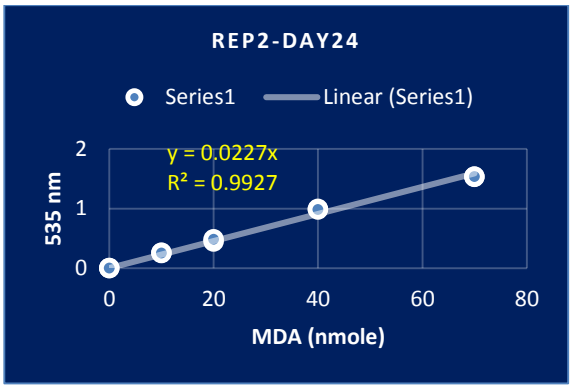


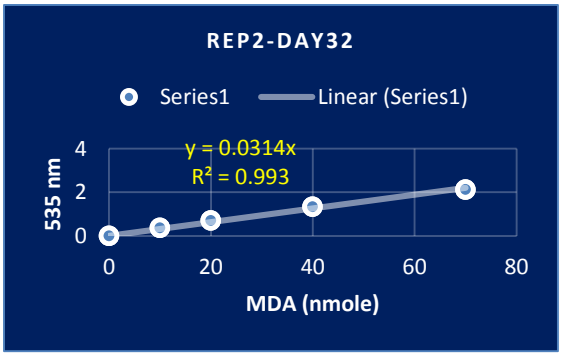
TBARS Standard Curves – Second Replication

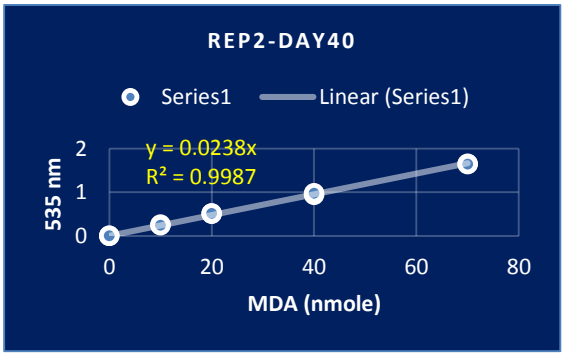


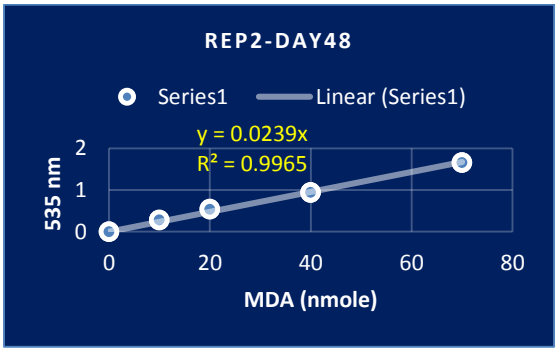




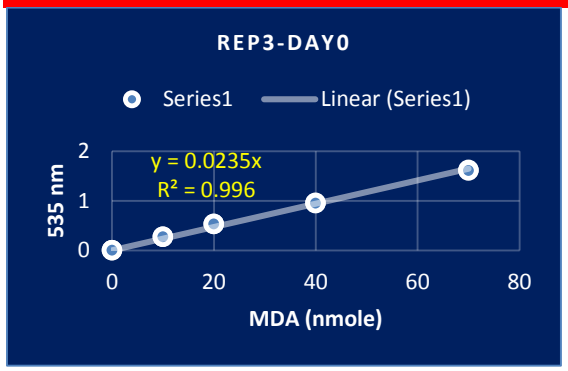


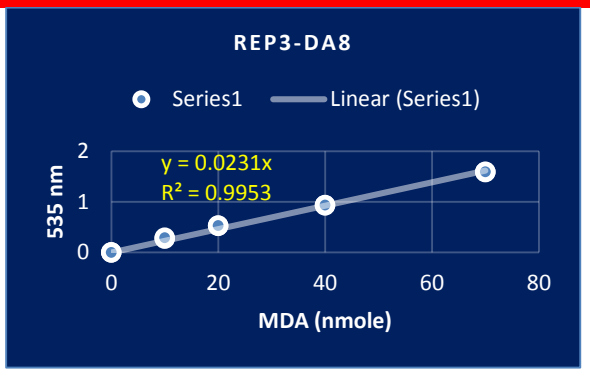


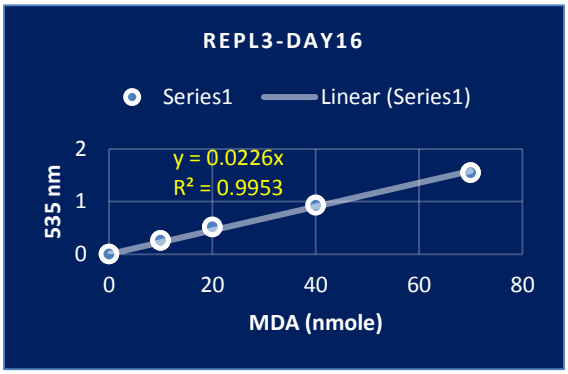


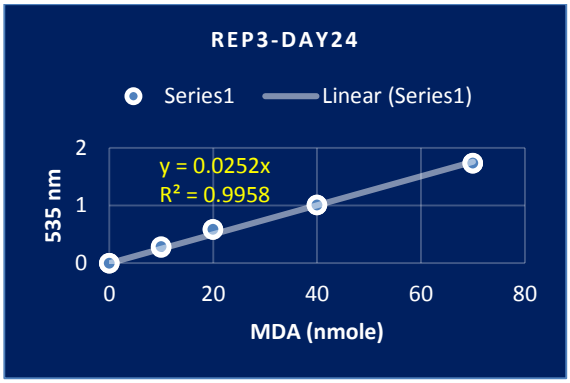


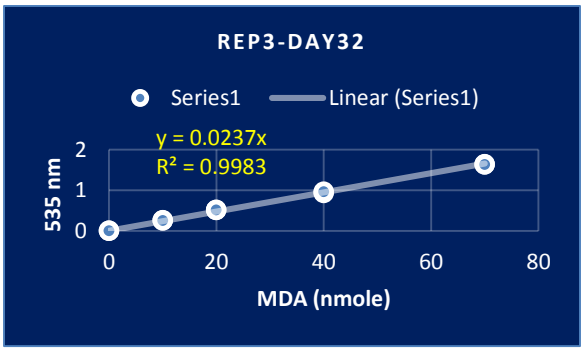
TBARS Standard Curves – Third Replication

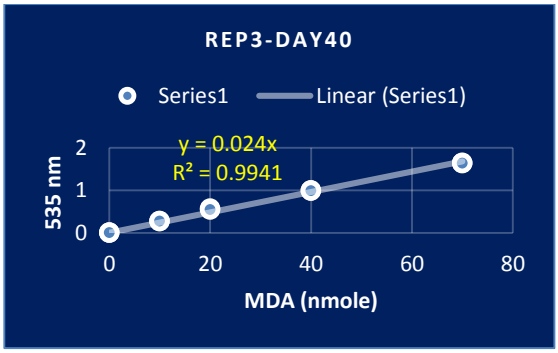


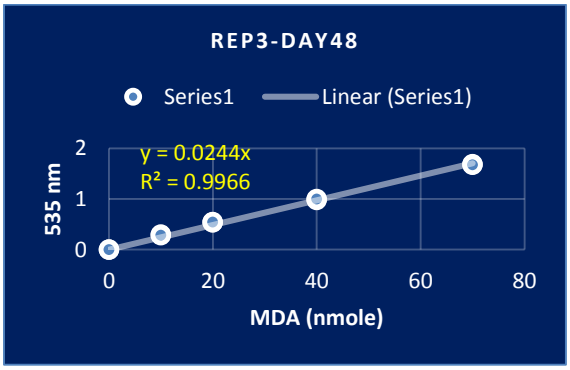




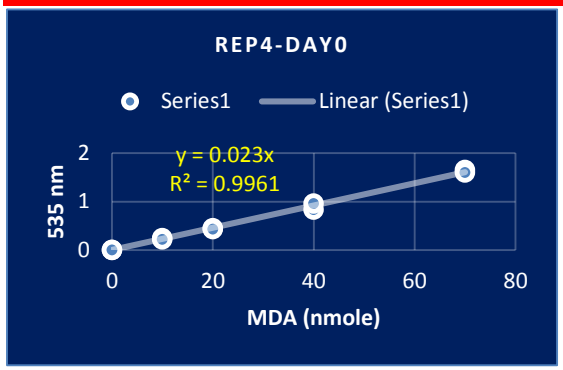


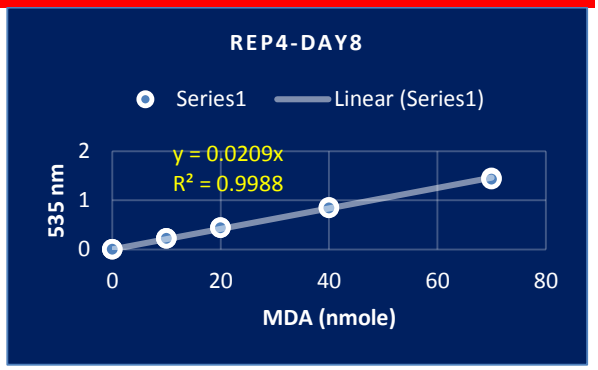


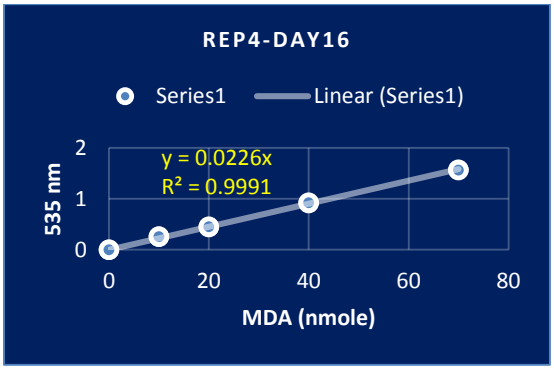


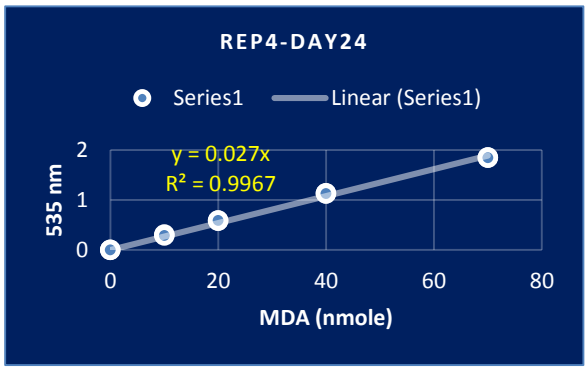


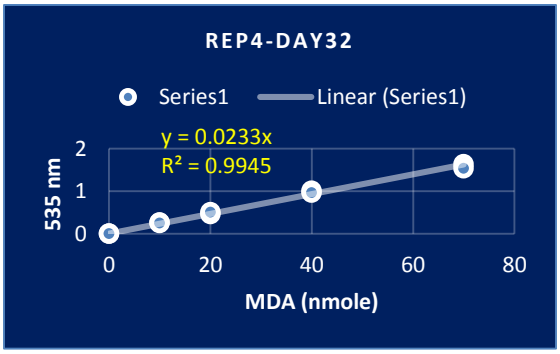
TBARS Standard Curves – Forth Replication

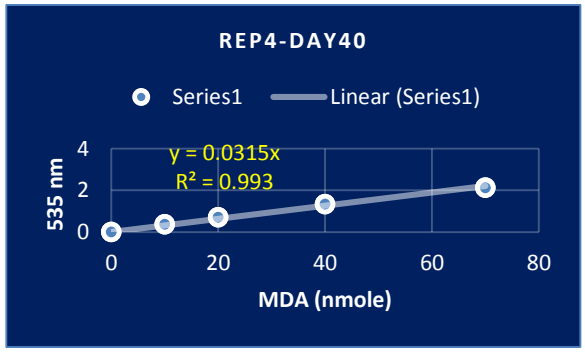


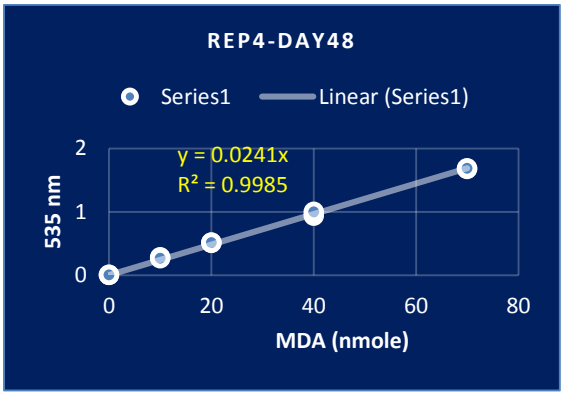












TBARS Standard Curves – Fifth Replication