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Oxidative stability evaluation of milk from cows fed dried distillers grains with solubles, by sensory and chemical analysis

by

Gerui Li

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:

Stephanie Clark, Major Professor
Donald Beitz
Terri Boylston
Chong Wang

Iowa State University

Ames, Iowa

2013

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ABSTRACT

The feeding of distillers dried grains with solubles (DDGS) to dairy cows has been loosely implicated in formation of oxidized off-flavors in milk. The purpose of this study was to examine the impact of feeding DDGS to dairy cows on the oxidative quality of milk by sensory and chemical analysis. Twenty-four cows were divided into two groups, fed a total mixed ration, with three incorporation levels of DDGS (0% (control), 10%, 25%) in a two-group three-period crossover design. Each group received each of the diets, such that each cow served as her own control. Milk was collected on days 14, 21, 28 during each of the experimental periods. For each diet treatment, pooled fresh milk was HTST pasteurized then divided into three fortification groups (no vitamin addition (control), 0.06% Vitamin E, 0.06% Vitamin C). Milk fat (%), SNF (%), protein (%) were measured using LactiCheck™. A 10-member descriptive analysis panel evaluated the milk samples on seven specific descriptors on days 1, 3, 7 of storage. Chemical analyses (peroxides, free fatty acids (FFA)) were conducted on the same milk with SafTest™ kits. Milk fat% were similar in 0% and 10% DDGS groups, while it was significantly ($p < 0.0001$) lower in the 25% DDGS group (2.6%); SNF% and protein% increased with the inclusion of DDGS ($p<0.05$). Sensory analysis revealed diet treatment, storage day, and fortification effects ($p < 0.05$) on oxidized off-flavors. Milk from 25% DDGS, Vitamin C fortification, or collection day 14 had higher off-flavor scores ($p<0.05$). Though statistically significant, the milks did not exhibit definite oxidized flavor; the scores were lower than 1.5 on a 15-cm line scale. All peroxide and most of the FFA measurements were below detection level, with the exception of a few

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samples that had slightly elevated FFA; the elevated results were not observed in their replicates. With no apparent oxidation in any milk from any treatment, the sensory and chemical analyses support the conclusion that feeding of DDGS at 10% and 25% levels did not decrease the oxidation stability of milk. Spontaneous oxidation is a complex process that cannot be blamed on DDGS alone.

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CHAPTER 1: INTRODUCTION

Milk is the mammary secretion from all mammals to feed and nurture the young. Through the history of mankind, milk has evolved from its primary function of nourishing the neonates to being part of the human food supply for all ages. Humans have been consuming large amounts of milk from non-human species for centuries, and cows' milk is one of the principal types (Wong et al., 1988). When people mention milk, they are generally referring to cows' milk. Cows' milk certainly plays an important role in providing energy and nutrients to humans. The FDA defines milk as the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows (CFR, 2012).

Milk Production And Consumption

In the United States, massive amounts of cows' milk are produced every year. Milk production has increased 18% over the past 10 years, from around 170 billion pounds in 2003, to about 200 billion pounds in 2012 (USDA-NASS, 2013). Of all the milk produced, about one third of the supply goes into fluid milk and cream products while the rest is used for a wide range of dairy products, such as cheese, butter, and frozen dairy products (USDA-ERS, 2012). Conventional plain fluid milk, whole, reduced fat 2%, low fat 1%, and nonfat skim milk, make up about 83% of the sales of all fluid milk and cream products (ERS, 2012). Even though the per capita consumption of fluid milk continues to decline, in 2011,

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people in the U.S. consumed 201 pounds of fluid milk and cream products per capita (ERS, 2012; USDA-ERS, 2012).

Milk Composition

Milk is a complex biological fluid. There could be as many as 10^5 different kinds of molecules in milk (Wong et al., 1988). The major constituents are water, fat, protein, lactose, vitamins, and minerals. The proximate analysis composition of milk, as summarized in four references, is shown in Table 1. From the physiochemical structure point of view, milk is a solution of lactose, salts, and other small molecules in water, with colloidally dispersed proteins, and emulsified lipids in the form of fat globules (Harding, 1995).

Table 1. Proximate analysis of milk composition from various references

Sources / Milk Constituents	Jenness and Sloan, 1970	Herrington et al., 1972	Fox and McSweeney, 1998	Jensen, 1995
Water, %	87.3	87.8	87.4	87.35
Fat, %	3.9	3.53	3.7	3.6
Protein, %	3.25	3.13	3.4	3.36
Lactose, %	4.6	4.82	4.8	4.7
Ash, %	0.95	0.72	0.7	0.99

Milk constituents

Water is the most abundant constituent in milk, at around 87 to 88%, and gives milk a water activity of 0.993 (Wong et al., 1988). Water acts as the solvent for the constituents in milk.

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The lipids in milk are sometimes referred to as 'butterfat'. The primary purpose of the lipids is to provide energy to the newborns (O'Connor and O'Brien, 2006). Milk lipids are the source of essential fatty acids (FAs) and fat-soluble vitamins; they also contribute to the rheological properties and flavor characteristics of dairy products (Fox and McSweeney, 1998). Milk fat content can vary from about 3% to 6%, but it usually stays in the range of 3.5% to 4.7% (O'Connor and O'Brien, 2006). Almost all the milk fat exists in the form of fat globules with surface milk fat globule membranes on the outside (Wong et al., 1988). There are a number of different lipids in milk. The majority of the milk lipids, about 98%, are triglycerides and they are mostly found in milk fat globules (Walstra and Jenness, 1984). About 0.5% to 1% of the lipids are phospholipids, which play an important role in the milk fat globule membrane (Jensen et al., 1991). Sterols, about 0.5% of the total milk lipids, are found in milk as well, most of which are cholesterol. Other lipids including monoglycerides, diglycerides, free fatty acids, and hydrocarbons, which also exist in milk but only in trace amounts (Jensen, 1995).

The protein in milk can be classified into several groups, including caseins, whey proteins, milk fat globule membrane proteins, some minor proteins, and enzymes (Wong et al., 1988). About 80% of proteins in milk are caseins. They are a group of phosphate-containing proteins that are specific to milk and are dispersed in milk as casein micelles (Walstra and Jenness, 1984). The other 20% are mostly whey proteins that are dissolved in solution in milk (Fox and McSweeney, 1998). One should note that proteins are usually measured by converting the nitrogen content of the milk into the crude protein content and

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about 5% of the milk nitrogen is non-protein nitrogen from ammonia, urea, creatinine, creatine, etc. (Cerbulis and Farrell, 1975; Fox and McSweeney, 1998).

Virtually the entire carbohydrate portion of milk is lactose. Only trace amount of other sugars, such as glucose, fructose, glucosamine, neutral and acidic oligosaccharides exist in milk (Fox and McSweeney, 1998). Lactose is unique and distinctive to milk. It is a reducing sugar composed of one molecule of glucose and one molecule of galactose.

The term "ash" is used to communicate the mineral content in milk; ash is composed the noncombustible components in milk. The principal milk salt constituents include calcium, potassium, sodium, chloride, and citrate. (Wong et al., 1988; Fox and McSweeney, 1998). Some other elements are found in trace amounts (Walstra and Jenness, 1984). Milk contains various vitamins that are essential for growth and maintaining biological functions. The vitamins in milk are classified into two groups based on solubility. Water-soluble vitamins are vitamin B groups, including thiamin, riboflavin, niacin, biotin, folate, B_6, and B_{12} and vitamin C, ascorbic acid. Most B group vitamins are co-enzymes or precursors of co-enzymes. Of particular relevance in the context of this thesis, milk is a good source of riboflavin and whole milk contains about 0.17mg of riboflavin per 100g of milk (Fox and McSweeney, 1998). Riboflavin is stable under pasteurization but is photodegradable under light, which is linked to the autoxidation of milk fat (Jensen, 1995). Fat-soluble vitamins are vitamin A, vitamin D, vitamin E, and vitamin K. They all have important functions in biological system. Vitamin A is crucial in the vision process; vitamin D acts as a hormone; vitamin K is a co-enzyme; and vitamin D is an effective antioxidant

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(Fox and McSweeney, 1998). In the United States, the Food and Drug Administration allows the optional fortification of vitamin A and/or vitamin D in whole milk (CFR, 2012).

Factors affecting milk composition

The composition of milk is not absolute. It is influenced by various factors and conditions (Jensen, 1995; Fox and McSweeney, 1998). For example, both fat content and fatty acids profile can be quite variable depending on the cow breed, feed, season, stage of lactation, etc. (Jensen, 2002). There are generally three kinds of factors: inherited (e.g. species, breeds, individuals), physiological (e.g. stage of lactation, age, heath), and environmental (e.g. season, climate, feed) (Walstra and Jenness, 1984). There is a profound breed effect on milk yield and less of an effect on milk composition. However, differences do exist among different breeds. For example, Holsteins tend to produce milk with lower fat content compared with Jerseys and Guernseys (Harding, 1995). The seasonal changes in milk composition can be attributed to the extremes in environmental temperatures. Milk fat and protein contents tend to be lower from cows under heat stress in the warmer months (Wong et al., 1988). Nutrition is a key factor affecting milk composition. Milk composition can be altered by manipulation of the feed based on the amounts and types of different mixes in the feed (Fox, 1983). For instance, a low intake ratio of roughage to carbohydrates will result in decreased fat content in milk (Grummer, 1991). Milking intervals, milking rates, milk frequencies, and milking routines also can influence the compositional outcome of the milk (Harding, 1995; Klei et al., 1997). Ordinary fatty acid changes in feed have little effect on the fatty acids composition of the milk, because

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extensive biohydrogenation by the microbes will occur in the rumen (Fox, 1983; Jensen et al., 1991). Changes in milk fatty acid composition have been observed with dietary manipulation (Palmquist et al., 1993; Grummer, 1991).

Milk fatty acids profile

Milk lipids are considered to be one of the most complex natural fats and oils systems (O'Connor and O'Brien, 2006). Milk contains a wide range of different fatty acids. More than 400 different fatty acids have been detected in milk (Christie, 1995). However, only a few of the fatty acids are the principal fatty acids in milk and the majority of the fatty acids are only present in trace amounts (Fox and McSweeney, 1998). Milk fatty acids can come from two sources, from the plasma lipids that originated from diet or adipose tissue, and *de novo* synthesis in the mammary gland (Fox, 1983; Lindmark-Månsson, 2008). About 45% of the fatty acids come from *de novo* synthesis, while the rest come from plasma lipids (O'Connor and O'Brien, 2006). These two sources also provide different fatty acids to milk. *De novo* synthesis supplies short-chain and medium-chain fatty acids and some C_{16} fatty acid, while some C_{16} and long-chain fatty acids, such as C_{18}, come from the plasma lipids (Fox and McSweeney, 1998). To analyze the milk fatty acids profile, the fatty acids are usually methylated or butylated, to be released from milk lipids, and then the fatty acid esters can be analyzed on GC for identification and quantification (Wong et al., 1988). The fatty acid composition is summarized from several articles and presented in Table 2; the fatty acid profiles are quite variable depending on the source.

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Table 2. Milk fatty acids composition from various reference sources

Sources / Fatty Acids*	Bitman and Wood, 1990	Jensen et al., 1991	Jensen, 1995	Wong et al., 1988	Christie, 1995
$C_{4:0}$	3.79	1.61	3.32	3.25	3.3
$C_{6:0}$	2.41	1.90	2.34	2.32	1.6
$C_{8:0}$	1.44	1.30	1.19	1.85	1.3
$C_{10:0}$	3.49	3.25	2.81	4.02	3.0
$C_{12:0}$	4.61	3.66	3.39	4.15	3.1
$C_{14:0}$	12.76	11.28	11.41	11.05	9.5
$C_{14:1}$	1.64	1.34	2.63	0.47	-
$C_{15:0}$	1.65	1.38	1.48	0.95	-
$C_{16:0}$	43.74	32.31	29.53	26.15	26.3
$C_{16:1}$	2.62	3.55	3.38	1.28	2.30
$C_{17:0}$	1.39	1.11	0.60	0.70	-
$C_{18:0}$	11.26	7.82	9.84	9.6	14.6
$C_{18:1}$	11.26	22.44	24.1	25.74	29.8
$C_{18:2}$	1.63	2.59	2.78	3.19	2.4
$C_{18:3}$	0.22	0.91	1.13	0.62	0.8

*Fatty acid contents expressed as g per 100 g of total fatty acids.

In general, the fatty acids can be sub-categorized into saturated fatty acids, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and trans fatty acids. Saturated straight-chain fatty acids, carbon length ranging from 4 to 18, account for about 70 to 75% of the total fatty acids (O'Connor and O'Brien, 2006). The most significant saturated fatty acid, from a quantitative point of view, is palmitic acid ($C_{16:0}$), which makes up about 25 to 30% of the total, while $C_{14:0}$ and $C_{18:0}$ are in the range of about 10 to 13% (O'Connor and O'Brien, 2006, Wong et al., 1988). Odd chain fatty acids also can be found in very low concentration in milk, such as pentadecanoic acid (C_{15}) and heptadecanoic acid (C_{17}). They are not synthesized in the mammary gland but in the rumen by bacteria and then transferred into plasma lipids (Lindmark-Månsson, 2008). In some articles, high percentages of short-chain fatty acids, $C_{4:0}$ and $C_{6:0}$ are reported in milk lipids. This is

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because they were expressed in mole percentages (Walstra and Jenness, 1984). All the percentages in this review will be expressed as weight-base percentages, unless specified.

Monounsaturated fatty acids make up about 18 to 24% of the total milk fatty acids, most of which is oleic acid ($C_{18:1}$). It is the most abundant unsaturated fatty acid in milk. Small amounts of $C_{14:1}$ and $C_{16:1}$ are present in milk fat as well (Jensen et al., 1991). Polyunsaturated acids are in very low concentration in milk because of the biohydrogenation in rumen. Linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) are the major polyunsaturated fatty acids in milk. They are essential acids that can only be supplied by the diet (Wong et al., 1988; Jensen, 2002). Cows' diet seems to affect the concentration of linolenic acid in milk fat; it is higher in milk from pasture-based cows than from barn-fed cows (Ellis et al., 2006). Trans fatty acids are naturally occurring fatty acids in milk. Approximately 2.5% of the fatty acids in milk are trans fatty acids, with vaccenic acid (t11-$C_{18:1}$) being the major one (Jensen, 1995; Lindmark-Månsson, 2008). Some other minor acids, such as keto (oxo) and hydroxyl fatty acids, and branched fatty acids have been detected in milk as well (Wong et al., 1988; Jensen, 1995; Jensen, 2002).

Because of the wide range of fatty acids, milk fat has the unique dairy flavor that people enjoy. However, these lipids sometimes serve as substrates and precursors for the hydrolytic and oxidative rancidity products that contribute to off-flavors in milk; milk fat can also act as a solvent for off-flavor compounds from the surroundings (Fox and McSweeney, 1998).

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Milk Taste And Off-Flavors

One of the most important qualities of a food is its taste (Walstra and Jenness, 1984). Milk has a neutral, clean, pleasantly sweet flavor profile (Clark et al., 2009). It should not have a foretaste or an aftertaste other than the natural dairy richness from milkfat and other milk solids (Bodyfelt et al., 1988). However, milkfat not only serves as the solvent for many flavor compounds but also as the origin of may flavors, including some off-flavors. And the relatively bland and mild taste of milk makes it very susceptible to a variety of flavor defects (O'Connor and O'Brien, 2006). In general, off-flavors in milk can be categorized into four groups (A, B, C, D): absorbed, bacterial, chemical, and delinquency (Clark et al., 2009). Absorbed off-flavors are those absorbed from the environment. Some absorbed off-flavors include barny, cowy, feed, garlic/onion. Bacterial off-flavors are caused by the bacterial degradation of the milk, such as acid, bitter, malty, and rancid. Chemical off-flavors come from the chemical changes within the milk caused by processing and storage conditions. Cooked, light/metal oxidized and lacks freshness are all in this category. Delinquency off-flavors are those that result from a person making a mistake due to inattention, such as flat (water), foreign (sanitizer), and unclean (Clark et al., 2009). Out of all the off-flavors, oxidized flavor is probably the most common flavor defect in milk. It has received more research and quality control attention over the years than any other flavor defect, yet it still seems be to problematic in the dairy industry (Bodyfelt et al., 1988). Oxidation of unsaturated milk fat produces unstable hydroperoxides, which give rise to a wide variety of carbonyl products, many of which can be attributed to the oxidized off-flavor in milk (O'Connor and O'Brien, 2006). In 1978, the Committee on Flavor

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Nomenclature and Reference Standards of the American Dairy Science Association published an extensive bibliography and classified the descriptive terms of the oxidized flavor as "papery," "cardboard," "metallic," "oily," and "fishy," (Shipe and others, 1978). As this is the focus of this thesis, subsequent sections will elaborate on this milk flavor defect.

Milk Lipid Oxidation

Lipid oxidation is one of the most basic chemical deterioration reactions in foods. It can result in undesirable sensory properties and loss of nutritional quality (Coupland and McClements, 1996). The mechanism of lipid oxidation has been studied extensively through the years. The hydroperoxide theory of the oxidation of unsaturated fatty acids is universally accepted (O'Connor and O'Brien, 2006). Lipid oxidation is a free radical-catalyzed chain reaction that involves three steps: initiation, propagation, and termination. The general lipid oxidation reaction formulas are shown in Figure 1. Unsaturated fatty acids are firstly oxidized to form lipid hydroperoxides, which then break down into various carbonyl and other compounds that cause the oxidized off-flavor in milk (Wong et al., 1988).

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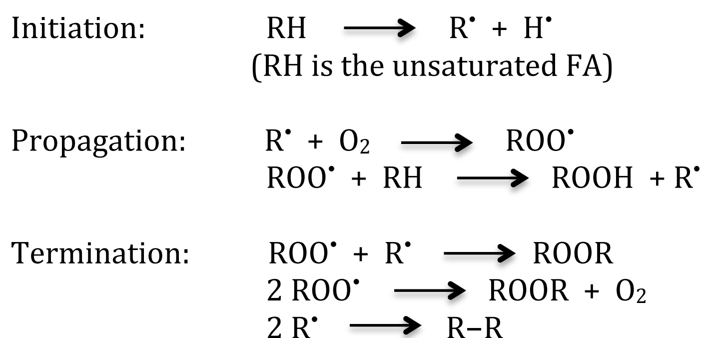

Figure 1. The three steps (initiation, propagation, and termination) involved in lipid oxidation reaction

The first step in lipid oxidation is initiation. It is the removal of a hydrogen atom ($H^\bullet$) from the unsaturated fatty acid to form a fatty acid free radical ($R^\bullet$). The hydrogen atom is abstracted from the methylene group adjacent to the double bond in the unsaturated fatty acid. Although it may be possible for saturated fatty acids to lose an $H^\bullet$ and undergo oxidation, lipid oxidation principally only involves unsaturated fatty acids, especially polyunsaturated fatty acids. Because the hydrogen atoms on the methylene groups in unsaturated fatty acids are much easier to disassociate than in saturated fatty acids, the ease of methylene hydrogen disassociation increases as $C_{18:0} < C_{18:1} << C_{18:2} << C_{18:3}$ (Fox and McSweeney, 1998). There are a number of factors that can trigger this reaction, including light (particularly UV lights), transition metal ions, irradiation, enzymes, and active oxygen species (Coupland and McClements, 1996; O'Connor and O'Brien, 2006).

Then the resulting free radicals ($R^\bullet$) react with ground state oxygen (O_2) to form lipid peroxide free radicals ($ROO^\bullet$) in the propagation step. The formed peroxide free radical will then, in turn, react with another molecule of unsaturated fatty acid (RH) to form lipid hydroperoxide (ROOH) and turn the unsaturated fatty acid (RH) into another

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unsaturated fatty acid free radical ($R^{\bullet}$), hence, continuing the chain reaction. Many different peroxides/hydroperoxides can be formed during the initiation and propagation steps of lipid oxidation. Because the C-H bonds of the methylene groups adjacent to the double bond(s) have different amounts of energy, different hydrogen atoms can be lost during the initiation step, as shown in Figure 2. Hence, different unsaturated fatty acid free radicals can be formed. Plus formed free radicals are stabilized by their own resonances. So when the unsaturated fatty acid free radicals react with oxygen, multiple isomeric lipid peroxide free radicals will be generated from each kind of unsaturated acid. Oleic acid can give rise to four isomeric hydroperoxides, while linoleic acid is capable of generating seven, and linolenic acid can generate ten possible isomeric hydroperoxides. Besides the formation of hydroperoxides, other compounds, including polyperoxides, epoxides, and cyclic peroxides, can be formed during the propagation step as well (Wong et al., 1988). Fatty acid oxidation always has to go through the intermediate step of peroxides/hydroperoxides formation. Schaich has proposed that the rate of oxidation is directly proportional to the amount of peroxide produced (Schaich, 1980).

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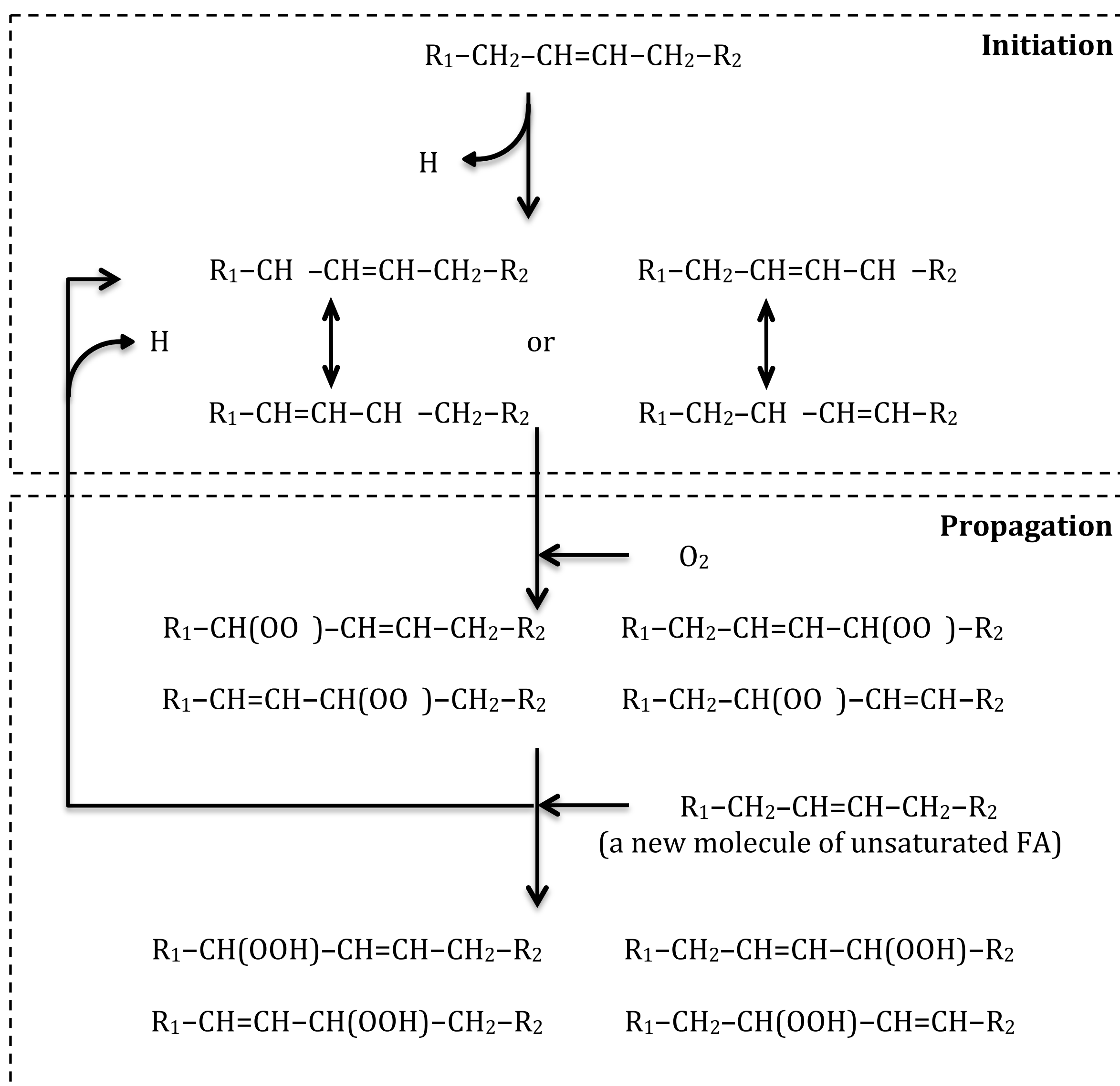

Figure 2. Mechanism for the formation of various isomeric lipid hydroperoxides from a single fatty acid

The hydroperoxides are unstable and readily decompose into a wide range of carbonyl products. The main products include saturated and unsaturated aldehydes, saturated and unsaturated ketones, saturated and unsaturated hydrocarbons, semi-aldehydes, and saturated and unsaturated alcohols. The many minor unsaturated fatty acids in milk can go through the same oxidation steps and create more different carbonyl

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products (O'Connor and O'Brien, 2006). Milk oxidation products are extremely complex. Many of these carbonyl products are the compounds that are responsible for the oxidized off-flavor in milk. There are great challenges to correlate specific off-flavors in milk with specific carbonyls or groups of carbonyls. The multitude of the compounds must first be generated, then the quantification of each compound, the differences in thresholds, the possible additive or antagonistic effects, the existence of unidentified compounds, and the interaction with the milk matrix, all contribute to the difficulty and complexity of oxidized off-flavor in milk (Wong et al., 1988).

Lipid oxidation in milk is nearly the same as in pure lipids. However, milk is an oil-in-water emulsion; many antioxidants and pro-oxidants in the aqueous phase will contribute to the oxidative stability of milk lipids (Coupland and McClements, 1996). Hence, oxidation in milk is more complex than ordinary lipid oxidation. There are many factors, both intrinsic and extrinsic, that influence the rate and extent of lipid oxidation in milk. The degree of unsaturation is certainly one of the principal factors influencing the oxidative stability of milk (O'Connor and O'Brien, 2006) along with oxygen species and availability, light, metals, enzymes, tocopherols, ascorbic acid, and thiols (Waraho et al., 2011).

Milk rich in unsaturated fatty acids has increased susceptibility to oxidation (Palmquist, 1993). Studies have shown that a high concentration of linoleic acid in milk seems to be associated with increased development of oxidized flavor in milk (Barrefors et al., 1995; Grandelli et al., 1998). Oxygen will certainly have great impact on oxidation since it is a substrate of the reaction. Many studies have shown that light (particularly UV light) is

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very effective in promoting lipid photooxidation in milk (Singleton, 1963; Mortensen, 2000; O'Connor and O'Brien, 2006). Light can excite the hydrogen and promote the formation of unsaturated fatty acid free radicals in the initiation step of lipid oxidation. The water-soluble vitamin, riboflavin, is a very potent photosensitizer. It can catalyze the photooxidation of lipid in milk (Fox and McSweeney, 1998) and it has also been reported that riboflavin generates reactive oxygen species, such as peroxide anion, in milk when exposed to UV light (Kim, 2007). Transition metals, such as iron and copper, are major pro-oxidants in food emulsions like milk. They are capable of catalyzing the decomposition of hydroperoxides (Yoshida and Niki, 1992; Dimakou et al., 2007). The metals may be indigenous, as part of the xanthine oxidase or lactoperoxidase system, or may be from contamination of equipment, water, soil, etc. (Fox and McSweeney, 1998). However, metal oxidation by equipment is rare nowadays, because of the utilization of stainless steel in dairy processing.

Antioxidants work against oxidation by scavenging free radicals and/or inactivating pro-oxidants (Waraho et al., 2011). They are molecules that have readily detachable hydrogen atoms. They can donate the hydrogen atom to lipid free radicals or lipid peroxide free radicals to stop the chain reaction from proceeding further, while the antioxidant residuals remain stable in milk (Fox and McSweeney, 1998). There are various antioxidants in milk, enzymatic and non-enzymatic. Different enzymes can prevent the formation of free radical or scavenge free radicals and peroxides/hydroperoxidizes, while some other enzymes are capable of catalyzing the synthesis or regeneration of non-enzymatic antioxidants (Lindmark-Månsson and Åkesson, 2000). Some non-enzymatic antioxidants

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include lactoferrin, vitamin E, vitamin C, carotenoids, thiol groups from proteins, and some products from Maillard reaction (Wong et al., 1988). Ascorbic acid, vitamin C, is a very effective alkoxyl free radical scavenger (Frankel, 1998), and as a water-soluble vitamin it functions as the antioxidant in the aqueous phase of milk. However, under certain condition ascorbic acid can possess pro-oxidative effect by regenerating the more pro-oxidative cuprous or perferryl radicals (Wong et al., 1988; Lindmark-Månsson and Åkesson, 2000). Vitamin E consists of eight vitamers, with α-tocopherol being the principal one in milk (Lindmark-Månsson and Åkesson, 2000). α-tocopherol functions as one of the major fat-soluble antioxidant in milk. It terminates the lipid free radical oxidation chain reaction by donating hydrogen or electrons to the free radicals and forms more stable products (Frankel, 1998). It has been reported that increased vitamin E content in milk increases the oxidative stability of the milk with higher unsaturated fatty acids (Focant et al., 1998).

Spontaneous Oxidation In Milk

In the early spring of 2009 and 2010, recurring consumer complaints about milk "going bad" in the Midwest drew the attention of farmers, processors, grocers, a major dairy cooperative, and educators. Experienced milk evaluators from those groups determined the primary off-flavor related to the rejected milk to be "oxidized". Raw milk, initially good, "spontaneously" became oxidized within a couple of days after milking. Significant attention, including grocery tours, plant tours, milk analysis, and feed analysis, was paid to determining the source of the "spontaneous oxidation" (SO). It was speculated

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that the source was at the cow level, meaning not from metal or light exposure. This is not the first incident of spontaneous oxidation in milk. Spontaneous oxidation in milk has been reported as early as the 1940s (Corbett and Tracey, 1943). Bruhn and Franke (1971) found 38% of the milk samples from Los Angeles to be susceptible to spontaneous oxidation. Various parts of the world have had similar reports, and even in some well-managed, high-producing dairy herds (Barrefors et al., 1995; Grandelli et al., 1998). There is not a universal standard definition for SO, is it fully understood. Spontaneous oxidation has been classified as oxidation that spontaneously happens within 48 hours of milking (Dunkley and Franke, 1967). Various factors have been linked to the probable cause of SO, but it is suspected that one primary source of oxidation came from the cows' dietary nutrition (Manitoba Agriculture, Food and Rural Initiatives).

Corn Distillers Grains

With the growing interest in corn-based ethanol as an energy source, there has been continuing growth in feeding the by-product, distillers grains, to dairy cows, particularly in the Midwest (Schingoethe et al., 2009). The expansion of the corn ethanol industry is leading to the increase in distillers grain supply. In 2006, 4.5 billion gallons of ethanol were produced in the US. The production more than doubled to 10.2 billion gallons in 2009, which resulted in a supply of distillers grains of 26.5 million tons. The estimated US supply of dried distillers grains with solubles (DDGS) in 2011 was about 34 million tons, and it is projected to reach about 38 million tons in the next decade (Hoffman and Baker, 2010). Feeding distillers grains to dairy cows is not a novel thing (Schingoethe, 2001). Distillers

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grains have been fed to cattle for more than a century (Loosli et al., 1952). It was not until recent years that distillers grains became a popular economic alternative for animal feed (Mathews and McConnell, 2009). DDGS is a cost effective replacement for corn and soybean meal as the sources of protein and energy for dairy cows (Schingoethe, 2001; Hoffman and Baker, 2010).

Dried distillers grains with solubles (DDGS) are the major by-product of the corn ethanol distillation industry (Rausch and Belyea, 2005). Corn is about two thirds starch. The highly fermentable starch is converted into ethanol and carbon dioxide during the fermentation and distillation process. The corn is ground and mixed with yeasts, enzymes, and water as the mash. The mash is heated to convert the starch into ethanol and carbon dioxide. The ethanol is distilled off and the leftover is separated into the liquid portion and the solid portion. The solid portion can be further dried to yield dried distillers grains, while the liquid portion gets condensed into distillers solubles. Since the condensed distillers solubles contain some nutrients, such as protein and vitamins, and provide some energy, it is combined with the dried distillers grains to produce DDGS (Mathews and McConnell, 2009).

DDGS contain most of the fiber, fat, protein, and minerals from the original corn after the starch was converted into ethanol. The nutrient content of DDGS can be quite variable based on the corn and the ethanol production process (Liu, 2011). Various publications have reported the general composition of DDGS, as shown in Table 3 (Spiehs et al., 2002; Schingoethe et al., 2009; Liu, 2011). The ~30% protein content makes DDGS a good source of protein and energy, while the ~10% lipid content and the ~41% readily

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digestible fiber (neutral detergent fiber) contribute to the high energy content of DDGS (Schingoethe et al., 2009).

Table 3. Basic composition of DDGS

Content	Amount (DM%)*
Moisture	9-12%
Crude Protein	29-31%
Lipids	10-12%
Total Carbohydrates	52-56%
Crude Fiber	~9%
Neutral Detergent Fiber	39-43%
Acid Detergent Fiber	16-18%
Ash	~5%

*Adapted and summarized from Spiehs et al., 2002; Schingoethe et al., 2009; Hoffman and Baker, 2010; Liu, 2011

Various studies have suggested that supplementing distillers grains to dairy cows could maintain or enhance their lactation performance; however, they have also observed a linear increase of unsaturated fatty acids in milk with the inclusion of distiller grains (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006). Because the fat in corn DDGS is quite unsaturated, with typically more than 60% linoleic acid ($C_{18:2}$), it should be expected to contribute an increase in unsaturated fatty acids in the milk (Schingoethe et al., 2009), despite the biohydrogenation by rumen microbes (Fox, 1983; Jensen et al., 1991). Hence, it is reasonable to suspect such increase in unsaturation of the fatty acids in milk could lead to the development of oxidation in milk from cows fed DDGS.

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Hypothesis

We hypothesize that higher level of DDGS feeding of cows will contribute to the development of spontaneous oxidized flavor (SOF) in milk. Limited DDGS feeding of cows, alone or in combination with vitamin E and vitamin C fortification of milk, will limit SOF in milk.

Objectives

The organoleptic property of a food is a principal purchasing criteria for consumers. Consumers are sensitive to off-flavors in their foods, particularly in mild flavored foods, such as milk. To maintain the highest quality of dairy products, we must be able to identify the problem and its source, and provide solutions to such problems. Understanding the relationship between feeding DDGS and subsequent SOF in milk will not only help dairy farmers to produce higher quality raw products, but also ensure high quality dairy products to increase consumer acceptability, and increase sales of dairy products.

Many distillers grains research projects have focused on the impact on dry matter intake, ruminal condition, feed efficiency, milk production, milk and milk fat composition (Schingoethe et al., 1999; Anderson et al., 2006; Schingoethe et al., 2009), but information is lacking in regards to milk sensory quality and/or susceptibility to oxidation. To our knowledge, the current study is the only study that evaluates the effect of DDGS feeding on the milk sensory quality by a descriptive analysis panel.

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The objectives of this study were:

- To evaluate milk sensory quality by a descriptive analysis panel.
- To conduct chemical tests to evaluate milk oxidative stability.
- To demonstrate the impact of vitamin E and vitamin C fortification upon SO of milk by sensory evaluation and chemical analysis.
- To assess the effect of DDGS feeding on SO of milk by sensory evaluation and chemical analysis.

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CHAPTER 2: MATERIALS AND METHODS

Experimental Design

The study was conducted from July to October 2011 at Iowa State University. Two groups of 12 mid-lactation Holstein dairy cows fed were three DDGS diets in a three-period two-group crossover design (Table 4). Thus, each cow received all three diets in different periods and served as its own control. The three diets were formulated to be isoenergetic with 0% DDGS (as the control), 10% DDGS (dry matter basis) incorporated, and 25% (dry matter basis) DDGS incorporated. Compositions of the diets were analyzed by Dairyland Laboratories, Inc. (Arcadia, WI) in each experiment period, as presented in Appendix A. The cows were introduced to their designated diets a week prior to the beginning (Day 0) of each period. The experiment periods officially started after the one-week diet acclimation time and the period lasted 28 days.

Table 4. Three dried distillers grains with solubles (DDGS) diets in a three-period, two-group crossover design

	Period 1	Period 2	Period 3
Group 1	0% DDGS	10% DDGS	25% DDGS
Group 2	10% DDGS	25% DDGS	0% DDGS

Milk from each group was collected on 14, 21, and 28 days after the official start of each period. The milk collected on each day from each group was then divided into three fortification portions: no fortification (control), 0.06% (w/w) vitamin E fortification, and 0.06% (w/w) vitamin C fortification. The milk was high temperature short time (HTST)

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pasteurized and stored for 1, 3, and 7 days. The experiment design scheme is shown in Figure 3.

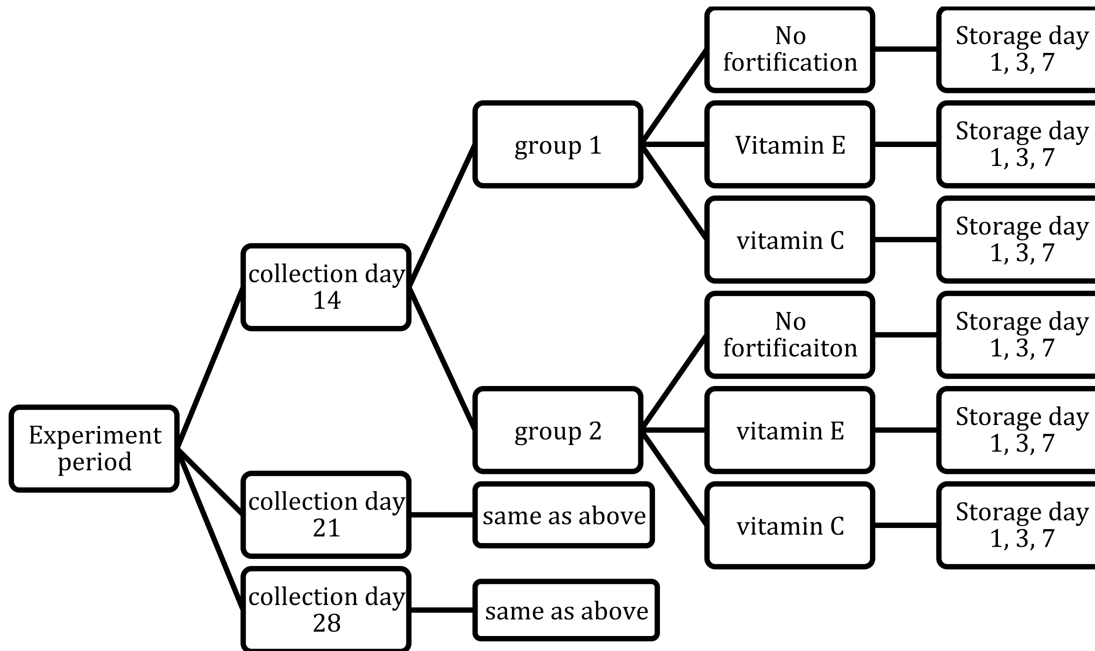

Figure 3. Experimental design flow chart for milk sampling for each experiment period

Milk Collection And Processing

During each experiment period (period 1, 2, and 3), milk was collected on each of the collection days (day 14, 21, and 28) at the Iowa State University (ISU) Dairy Farm. The ISU Dairy Farm milks cows three times a day. Because of scheduling and practicality, only the 9 am morning milking milk was collected and used in the study. Milk, not collected and used in this study, went into the ISU Dairy Farm bulk tank with the rest of the milk from the

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Farm. Before each collection, all the supplies (e.g. stainless steel milk cans, dump buckets, bucket caps, tubes, milking inflations) were cleaned with Ecolab® Oasis Enforce (St. Paul, MN) and sanitized with Ecolab® Mikroklene® sanitizer (St. Paul, MN). During milking, cows were milked one group (12 cows in each group) at a time. Milk was first collected in the dump buckets and pooled into three stainless milk cans for transporting. Dump buckets were rinsed with potable tap water before collecting milk from the other group. The collected milk, stored in cleaned and sanitized stainless steel milk cans, was then transported immediately after milking to the Center for Crops Utilization Research (CCUR) pilot plant in the Food Sciences Building on the ISU campus for processing.

At the pilot plant, the exteriors of the milk cans were rinsed out with cold tap water to remove any dirt and cooled with the running tap water at the same time. Milk, in the clean milk cans, was then chilled in the walk-in refrigerator in the CCUR pilot plant at 4°C for about 30 minutes until further processing. For each group, milk was filtered through layered cheesecloth suspended over a metal mesh sieve, and weighed into a tared stainless steel milk can. The weighed milk was pooled in one vat within each group (group 1 or group 2). The pooled milk from each group was then divided into three portions. One portion was left as the non-fortified control milk; one portion had 0.06% (w/w) of vitamin E (tocopheryl acetate) (Dairy House, Fenton, MO) added in as the vitamin E fortified milk; and one portion had 0.06% (w/w) ascorbic acid (Jianshan Pharmaceutical Co., LTD, Pure L-ascorbic acid, Jiansu, China) added in as the vitamin C fortified milk.

All six of the milks, three fortification milks (control, vitamin E, and vitamin C) from the two groups (group 1 and group 2), were HTST pasteurized continuously with a

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UHT/HTSTLab Electric Model 25HV Hybrid pasteurizer (MicroThermics®, Raleigh, NC) in the CCUR pilot plant. Milk from the lower DDGS% diet group was pasteurized first. Within each group, milk was pasteurized in the order of no fortification, vitamin E fortification, and then vitamin C fortification. Milks were pasteurized in such order so if any cross-contamination accidentally happened, it would not create as big of an impact on the samples. The in-between milks when switching milks at the pasteurizer inlet were discarded to ensure no cross-contaminated milk was collected. The pasteurization temperature was set at 74°C, and the flow rate was set at 5 L/min to ensure a holding time of 24 s at 74°C. The pasteurized milks were collected in commercial translucent plastic gallon milk jugs purchased from Anderson Erickson Dairy (Des Moines, IA). Three gallons of each of the six milks were collected, one gallon for each of the storage days (storage day 1, 3, and 7). All the gallon milk samples were tagged and labeled with an identification code, and then put into black opaque plastic bags to block light during transportation and storage. They were stored in a commercial refrigerator (Hobart®, Troy, OH) at 3°C in the CCUR test kitchen until analysis. A total of 162 one-gallon milk samples were obtained through the entire study. Each of them was given a unique random 3-digit code. No code was repeated in the study. These samples were used for the sensory evaluation and the oxidative stability test.

On the same collection days within each feeding cycle (collection days 14, 21, and 28), milk samples were collected from individual cows for milk proximate composition analysis. About 50-100 ml of milk sample from each cow was collected in sampling jars a Boumatic double-12 parallel milking system at the 7 pm evening milking on the same day

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as the 9 am morning collection. These milks were collected into amber opaque bottles (Fisherbrand, Fisher Scientific, Fair Lawn, NJ) labeled with each cow's unique identification number and transported in ice-bath coolers from the dairy farm to the chemistry laboratory in ISU Food Sciences Building. All the milk samples were kept in the laboratory refrigerator at 4°C overnight and analyzed for proximate composition the next morning.

Proximate Analysis

Each individual cow's milk was analyzed for fat, solid non-fat (SNF), and protein content, using the LactiCheck™-01 RapiRead Milk Analyzer (Page & Pedersen Intl. Ltd., Hopkinton, MA). Milk samples, taken out of the refrigerator, were gently swirled in the bottles to achieve a homogenous sample. They should not be shaken, in order to avoid foaming, because the incorporated air will interfere with LactiCheck™ measurements. About 30 to 40 ml of the milk sample was transferred into a small beaker from the amber bottles. The milk samples were then tempered to about 22±2°C and set at the LactiCheck™-01 sampling port for analysis. The LactiCheck™-01 automatically takes milk sample from the sampling port and instantly displaces results of fat%, SNF%, and protein% for each milk sample. Duplicate readings were taken for each sample.

Sensory Evaluation – Descriptive Analysis

Panelist recruitment

The use of human subjects for the sensory panel of this study was approved by the Institutional Review Board of ISU in June, 2011. Recruitment flyers were posted around the

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campus at ISU. Also, recruitment emails were sent to the Food Science and Human Nutrition Department staff, faculty, and graduate students. The participants were required to be at least 18 years old, consume milk at least once a week, and have interest in sensory evaluation. The panelist selection was based on the participants' availability during the entire study. A total of 14 panelists were recruited for the study. Some had previous milk sensory evaluation experience and some had not. Ten people were selected as the official panelists. They were students and/or staff at ISU. The other 4 participants were from the study investigator's laboratory group. They served as backup panelists for whenever an official panelist had to miss an evaluation session or drop out of the study. Each panelist, including backup, was given $5 compensation for each training and evaluation session they attended.

Panelist training

The panelists received a total of 8 hours of training, 2 one-hour training sessions per week, over a 4-week period, before the experiment. Two additional one-hour review sessions were conducted between the experiment periods. Before the training sessions, 7 off-flavors (bitter, cooked, feed, flat, foreign, light oxidized, and metal oxidized) were selected by the investigators for the milk sensory evaluation in this study. They were chosen from the milk-scoring guide for the National Collegiate Dairy Product Evaluation Contest. These 7 off-flavor attributes were chosen because they were the expected possible off-flavors in the experimental milks. Any other off-flavors that were not listed or identified could be identified by panelists as "foreign", and be scored accordingly.

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During the first training session, the panelists were introduced to sensory evaluation and the purpose of this sensory panel. The current experiment was briefly explained to the panelists and consent forms were signed (Appendix B). The panelists were given a demonstration and instructions on the steps of milk tasting techniques. They were instructed to pour milk samples into clean disposable 3 oz. plastic cups (Solo cup company, Lake Forest, IL), and to fill about 1/4 to 1/3 of the cup (about 30-45 ml of sample). The panelists were told to 1) immediately cover the cup with one hand while holding the cup with the other hand to protect the milk sample from light and trap aromas inside the cup; 2) gently swirl the milk sample and use the heat of the hands to warm up the milk to release more volatile compounds; 3) take a deep sniff of the milk sample when lifting up the hand from the cup; 4) take a generous sip of the milk, roll it around the mouth, note the flavors and sensations, and then expectorate. The panelists were encouraged to breathe in fresh air through the mouth, and then exhale through the nose to enhance the aromas in the sample retronasally. A sample of "no defect" fresh whole milk (paper carton packaging) (Anderson Erickson Dairy, Inc., Des Moines, IA) from the local grocery store was presented to the panelists to familiarize them with the taste and sensation of fresh milk. A sample of light oxidized milk (regular translucent plastic packaging purchased straight off the shelf) (Anderson Erickson Dairy, Inc., Des Moines, IA) was given to the panelists after tasting the "no defect" milk sample to help them to identify and distinguish the "oxidized" flavor in milk. The panel leader guided the discussion among the panelists to describe the oxidized flavor. The light oxidized flavor was described as having a "cardboard" taste/aroma and a mouth-drying sensation.

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During the following 3 sessions, the panelists were introduced to the rest of the off-flavors (cooked, feed, bitter, metal oxidized, flat, and foreign). Milk samples for training were adulterated by the investigator to create each specific off-flavor (foreign was used for any flavor that was not supposed to be in milk and was not one of the other 6 off-flavors listed on the ballot; for the training session in this study, chlorine/sanitizer was chosen as an example for one of the possible foreign off-flavors). These sessions were set to familiarize panelists with each off-flavor and help them to recognize and identify each off-flavors in milk. During the sessions, 5 to 8 unidentified training milk samples were presented to the panelists, one at a time. After tasting one sample, the panelists would discuss their personal observations about that particular sample. Then the off-flavor in that sample would be revealed to the panelists. At this time, the panel leader would lead a discussion to help the panelists to recognize/remember the flavor/sensation of that off-flavor in milk. The goal was to establish a connection between the panelists' physical sensory responses and their cognitive recognitions of the particular off-flavors. Some flavor descriptors for off-flavors, as described by the panelists, are shown in Table 5.

After the first four training sessions, panelists were familiar with the off-flavors, so they were introduced to the scorecard for the study. The scorecard was used to score the intensity of each off-flavor using a 15 cm line scale. A training ballot (Appendix C) was used for the sessions. The intensity scores were adopted and modified from the milk-scoring guide for the National Collegiate Dairy Products Evaluation Contest. A "slight" was considered of a score around 3 cm on the 15 cm line scale; a "definite" was considered a score around 7.5 cm; a "pronounced" was considered of a score around 13 cm.

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Table 5. Some flavor/sensation descriptions for the seven off-flavors

Off-flavor	Descriptors
Bitter	Bitterness shows as an aftertaste; the taste was towards the back of the throat, piercing, throbbing
Cooked	Eggy, sulfur, custardy
Feed	Grassy, stalky, hay
Flat	Watered down, thinner mouth feel, less dairy fattiness
Foreign (chlorine/sanitizer)*	Bleach, swimming pool smell
Light oxidized	Cardboardy, pasty taste, mouth-drying sensation, smells like wet brown paper towel
Metal oxidized	Some similar characteristics as light oxidized, metallic tastes, penny coin taste, tingling sensation at the back of the tongue

*Foreign off-flavor is designated for off-flavors that are not supposed to be in milk but are not included in the other six off-flavors; during the training sessions, chlorine/sanitizer was chosen as an example of the possible foreign off-flavors.

The recipes and instructions for making the training milk are included in Appendix D. Similar to previous sessions, panelists were given unidentified samples for tasting, one at a time, which was followed by discussions about the off-flavor attribute and its intensity in that sample. The panel leader would then reveal the attribute and suggest the intended intensity score for that sample. The whole panel, including the panel leader, would discuss and come to an agreement on the attribute intensity. In particular, different levels of intensity for light oxidized and metal oxidized flavors were prepared for the training sessions (Appendix D). The panelists continued such training through session 5 to 6. The order of off-flavors on the ballot was also discussed. The panelists decided on the attributes to be listed in the order of cooked, feed, foreign, light oxidized, flat, bitter, and metal

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oxidized. It was based on the ease and the sequence that the off-flavors were detected by panelists.

During the last two training sessions, the panelists were brought to the Nutrition Wellness Research Center Sensory Evaluation Unit at Human Nutritional Science Building (HNSB) to familiarize themselves with evaluating milk samples in standard sensory booths with computerized ballots, which is how the real experiment milk samples would be evaluated.

Sample evaluation

The sensory evaluation of experiment milk samples was done with a sensory software Compusense® Five at the Nutrition and Wellness Research Center Sensory Evaluation Unit at HNSB on the ISU campus. Each panelist had a unique registration code to login the evaluation session on the computer. The evaluation ballot was divided into three pages in the program, with cooked, feed, and foreign scales on the first page; followed by a comment page for describing the foreign off-flavor (if any); then light oxidized, flat, bitter, and metal oxidized on the third page. The computerized ballot included 15 cm line scales for each off-flavor. The panelists would use the computer cursor to indicate the score (intensity) of each off-flavor for the sample. In each evaluation session, there were 6 experiment milk samples.

To prepare the samples, gallon containers were taken out of the refrigerator 30 minutes prior to the evaluation to take the chill off the milk. They were placed on the second shelf of a serving cart in a shaded area of the serving station in the sensory booth to

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avoid any direct exposure to fluorescent/sun light. About 30-45 ml milk samples were poured into pre-labeled (with the samples' 3-digit random number code) 3 oz. plastic cups on the spot during tasting. During the evaluation, the panelists were provided with plain tap water and unsalted saltine crackers as palate cleansers. Also, since "no defect" whole milk (Anderson Erickson Dairy, Inc., Des Moines, IA) was provided during training sessions as a reference for the panelists and the panelists prefer to have a "no defect" reference at the tasting sessions, a sample of "no defect" non-homogenized whole milk (Hansen's Farm Fresh Dairy, Hudson, IA) was provided to the panelists at sample evaluation. Non-homogenized whole milk was used here because milk samples in this study were not homogenized. The sample serving order for each panelist was pre-generated and randomized by Compusense® for each evaluation session. The panelists evaluated the samples on their own pace and could request more of any sample. However, they were not allowed to go back and change the score once they had finished evaluating one sample. All the sensory evaluation data were recorded in Compusense®.

Peroxide Value And Free Fatty Acid Content Analysis

To evaluate oxidative stability of the experiment milk samples chemically, peroxide value (PV) in milk was measured since peroxides are the primary lipid autoxidation product. Free fatty acid (FFA) content was also measured as another chemical measure of the milk's quality. Peroxide and FFA contents were measured using the SafTest™ system with ProxySafe™ STD kit and FaSafe™ STD kit (MP Biomedicals, Solon, OH). The same milk

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samples evaluated by the sensory panel were used for SafTest™. Milk samples were prepared following the SafTest™ sample preparation for STD assays protocol.

To prepare milk samples for assays, 1 ml of milk was transferred into the bottom of each conical tube; 1 ml of hexane (Fisher Scientific, Fair Lawn, NJ) and 3.5 ml of SafTest™ preparation reagent were added to each conical tube, then vortexed (Fisher Vortex Genie 2, Fisher Scientific, Fair Lawn NJ) for 1 minute on dial 8. After thoroughly mixed, the conical tubes were placed in the heat block for 15 minutes. The sample mixture was then filtered through a membrane filter (MP Biomedicals, Solon, OH) on the SafTest™ vacuum filtration unit. The clear filtrates were used for assays. Milk samples were tested in duplicate for peroxide and FFA assay. The analyses were conducted following the ProxySafe™ STD kit protocol and FaSafe™ STD kit protocol, which are available on the MP Biomedicals website (www.mpbio.com). The PV was reported as milliequivalents of peroxides per kilogram of sample (meq/kg) and the FFA content was reported as percent oleic acid in the sample (w/w).

Data Analysis

During the study, at any time, if a cow had become sick (e.g. mastitis), she was removed from that period of the experiment and returned for the next period when fully recovered. Replacement cows, when they were available, were used in some periods. In total, 25 cows were used in the study: 20 cows were in all three periods, 2 cows were in two periods, and three cows were in only one period of the experiment. Available data from all the cows were collected and used for analysis.

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Each off-flavor (sensory) score for each milk sample was reported as the average of the 10 panelists' scores, since variations among panelists were neither the focus nor the primary concern of the study.

All data analysis was performed in SAS 9.3 (Cary, NC). Fat%, SNF%, and protein% of the three diets (0%DDGS, 10% DDGS, and 25% DDGS) milks were analyzed using one-way ANOVA with Tukey-Kramer multiple pairwise comparison adjustment. Least squares means (lsmeans) procedure was used in the analysis to account for the unequal sample sizes. The means reported are weighted means. The primary concern regarding milk composition was the diet treatment. Hence, the diet treatment effect was the only experimental effect considered.

For the sensory data, a MIXED model with 5 fixed effects (diet treatment, collection day, fortification, storage day, and experiment period) and a random effect of cows (groups) was used. Tukey-Kramer adjustment was used for multiple pairwise comparisons. A significance level of $\alpha = 0.05$ was used to determine significant differences.

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CHAPTER 3: RESULTS AND DISCUSSION

Milk Proximate Analysis

Milk proximate compositions were analyzed nine times for each individual cow in the study (all data included in Appendix E1-E3). For simplicity of presentation, the fat, protein, and SNF contents were averaged (weighted means) for all cows over the 3 collection days (day 14, 21, and 28) for each diet treatment, in Table 6.

Table 6. Average fat, protein, and SNF contents of milks from the three DDGS diets

	0% DDGS	**10% DDGS**	**25% DDGS**
Fat% ± SE	3.17 ± 0.10[a]	2.89 ± 0.10[a]	2.60 ± 0.09[b]
Protein% ± SE	3.71 ± 0.03[a]	3.77 ± 0.02[b]	3.83 ± 0.02[c]
SNF% ± SE	9.90 ± 0.07[a]	10.02 ± 0.06[b]	10.19 ± 0.06[c]

[a, b, c] within the same row, significant differences exist when means do not share the same letter ($p<0.05$)

Mike protein and SNF contents were increased ($p<0.05$) by the dietary inclusion of DDGS (Table 6). However, such increases in protein and SNF were not observed by Nichols et al. (1998), Leonardi et al. (2005), Anderson et al. (2006), or Janicek et al. (2008). They all reported no differences in milk protein percentages between DDGS diet treatments and controls. One study did observe an increase in milk protein concentration when cows were fed high quality DDGS, and they suggested that depressed milk protein content could be an indicator of poor quality DDGS (Powers et al., 1995).

DDGS have about 11% (dry matter) lipid content (Hoffman and Baker, 2010). It is considered a good source of energy for dairy cows (Schingoethe et al., 2009). The primary purpose of including fats and oils in cow feed is to provide higher energy intake and to

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increase milk yield, however dietary lipid supplements could also affect the concentration of milk fat and the fatty acid composition in milk (Sutton, 1989). It has been well documented that increased level of lipid supplements in dairy cows' diet could lead to lower fat concentration in their milk (Palmquist and Jenkins, 1980; Charmley and Nicholson, 1994). The decrease in fat content has been attributed to the dietary lipids' effect on rumen fermentation (Kononoff, 2006) and the suppression of synthesis of short-chain fatty acids in the mammary gland (Offer et al., 1999).

Milk fat content of the control diet milk (3.17%) was similar to the 10% DDGS diet milk (2.89%) ($p=0.07$), but a decrease ($p<0.03$) in fat content in the 25% DDGS diet milk (2.60%) was observed compared with the control and 10% DDGS diet milks. Although statistically significant, the decrease in milk fat content in 25% DDGS milk was not detected by the sensory panelists (no differences in flat off-flavor detected) (Table 7). The control diet milk fat percentage was lower than expected, even for Holsteins, which usually have about 3.5% fat (Walstra and Jenness, 1984; Wong et al, 1988). A previous study also observed similar lower than expected fat content from Holsteins. They suggested it might be because of the high temperature of the environment, which may contribute to low milk fat concentration (Sasikala-Appukuttan et al, 2008); the current study was conducted from July through October.

In our study, 10% DDGS diet inclusion did not affect the milk fat content, while the 25% DDGS diet inclusion significantly decreased the fat content in milk. Typically, dairy nutritionists advise limitation of the dietary inclusion of DDGS because the dietary fat could contribute to milk fat depression (Pantoja et al., 1994). A study by Leonardi et al. (2005)

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found milk fat concentration was significantly decreased by the dietary inclusions of up to 15% distillers grains (Leonardi et al., 2005). Yet, Anderson et al. (2006) found no differences in fat contents among all diets (0%, 10%, and 20% DDGS); however they did observe a tendency of increased DDGS in cows' diet decreasing milk fat content (Anderson et al., 2006) and similar results were found by (Sasikala-Appukuttan et al, 2008).

Fatty Acid Profile

In the other part of the study, conducted by Eric Testroet, milk fatty acids were analyzed on individual cows (which is why the methods are not included in this document). The data are presented in Figure 3 because they are relevant to the findings in this study. The inclusion of DDGS in the cows' diet, at 25%, significantly decreased ($p<0.01$) the medium-chain fatty acids (C_8, C_{10}, C_{12}, C_{14}, and C_{15}) content and increased ($P<0.05$) the long-chain C_{18} fatty acids (oleic acid and linoleic acid) content in milk. No difference in $C_{18:3}$ was observed. Similar changes in fatty acids profile have been reported by many studies (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006; Sasikala-Appukuttan et al., 2008). Janicek et al. (2008) observed a linear increase in the trans-9 $C_{18:1}$ and $C_{18:2}$ content in milk with an increase in DDGS in cows' diet from 0 to 30%. The changes in milk fatty acid profile were attributed to the oil from the inclusion of DDGS (Leonardi et al., 2005).

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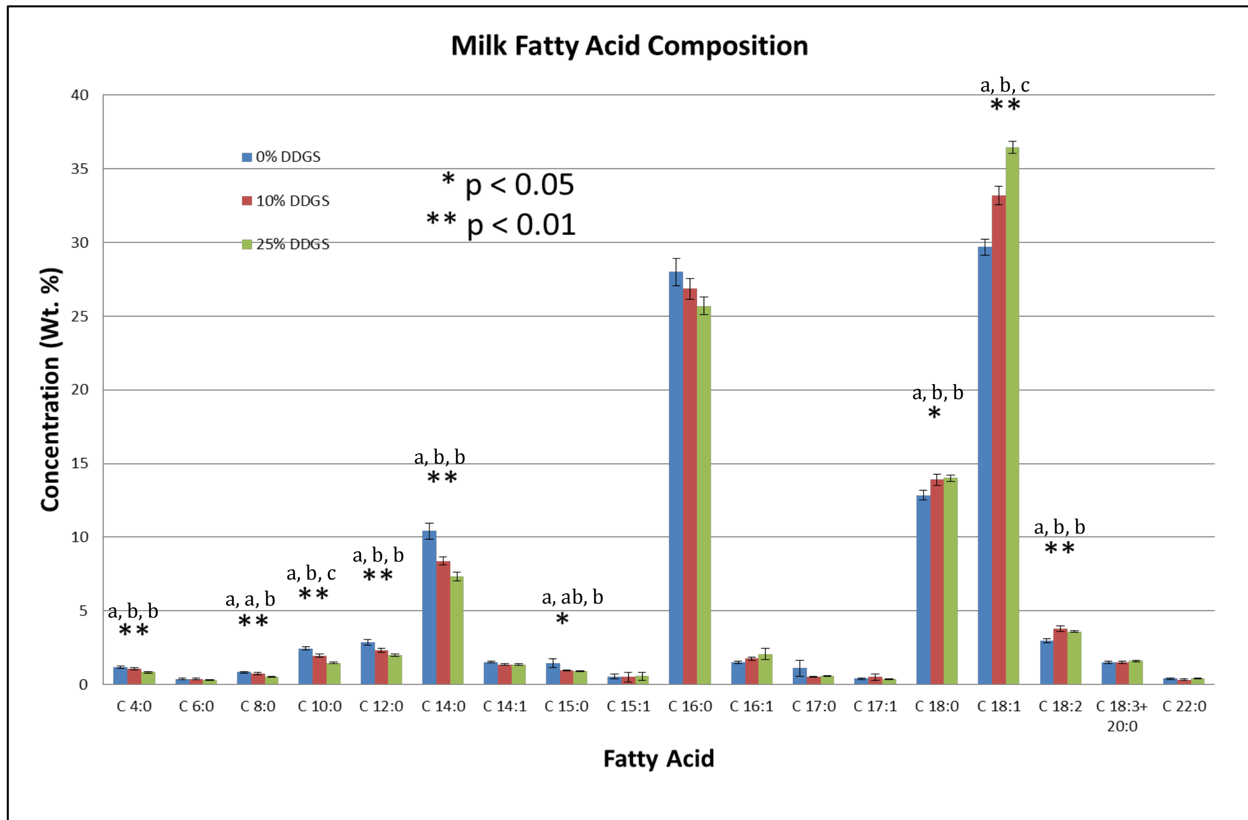

a, b, c statistical groupings for 0%, 10%, and 25% DDGS diet milks, significant differences exist when means do not share the same letter.

Figure 4. Milk fatty acids profile from all cows in comparison among three DDGS diets (from data collected by Eric Testroet at ISU, Ames, IA)

Sensory Evaluation

Sensory scores of each off-flavor for each fixed effect (diets, fortifications, collection days, and storage days) are organized and presented as the average scores from the 10 panelists in Appendix F1, F2, and F3. Each off-flavor was analyzed in a MIXED statistical model with fixed effects of diet, collection day, fortification, storage day, and experiment period. A summary table of the significances of each fixed effect on each off-flavor is presented in Table 7.

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The main experiment focus was to evaluate the diet treatment effect. In the sensory analysis, the diet treatment effect was only significant in metal oxidized flavor (Table 7). This result means that only metal oxidized flavor differed among the 3 DDGS diet milks. The 25% DDGS diet milk had significantly higher ($p<0.02$) metal oxidized flavor score than the control and 10% DDGS diet milks, while the control diet milk and 10% DDGS diet milk had similar scores ($p=0.95$) (Table 8). Scores of the 3 DDGS diet milks did not differ in any other off-flavors.

Collection day effect was significant from 4 off-flavors (cooked, feed, foreign, and light oxidized flavors) (Table 9). Milks from collection day 14 had significantly higher scores in cooked, feed, and foreign off-flavors ($p<0.02$) than collection day 21 and day 28. The collection day 14 and day 21 milks were not different ($p=0.14$) in light oxidized flavor, and the collection day 21 and day 28 milks were also not different in light oxidized flavor ($p=0.29$). But the day 14 milk had significantly higher ($p=0.03$) light oxidized score than the day 28 milk.

Fortification effect was significant in flat, metal oxidized, and light oxidized flavors. No fortification (control) milk had similar scores as the vitamin E fortification milk ($p>0.4$) from all 3 off-flavors, while the vitamin C fortification milk had highest scores from all 3 off-flavors (Table 10).

No storage day effect was observed; milk sensory scores did not differ in any of the storage days (day 1, 3, or 7), meaning no oxidation had developed through the 7 day storage.

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The experiment period effect was significant in feed, foreign, and flat off-flavors, however this was not a true complete evaluation of the trial effect since not all three diets (main experimental treatment) were administrated in each period. Also, the experiment period was not the study focus. So it is not further discussed.

Table 7. Summary demonstrating significance of each fixed effect on each off-flavor

	Diet	Collection day	Fortification	Storage day	Period
Cooked		*			
Feed		*			*
Foreign		*			*
Light Ox		*	*		
Flat			*		*
Bitter					
Metal Ox	*		*		

*The fixed effect was significant for that off-flavor ($p<0.05$)

Table 8. Mean metal oxidized flavor scores for the three DDGS diets

Diet treatment	Metal Oxidized Flavor
0% DDGS	0.49^{a}
10% DDGS	0.52^{a}
25% DDGS	0.79^{b}

$^{a, b, c}$ within the same row, significant differences exist when means do not share the same letter ($p<0.05$)

Table 9. Mean sensory scores of the four off-flavors for the three collection days

Attributes	Cooked	Feed	Foreign	Light Ox
Collection day 14	0.71^{a}	0.35^{a}	1.10^{a}	0.88^{a}
Collection day 21	0.41^{b}	0.22^{b}	0.51^{b}	0.75^{ab}
Collection day 28	0.36^{b}	0.19^{b}	0.61^{b}	0.66^{b}

$^{a, b, c}$ within the same row, significant differences exist when means do not share the same letter ($p<0.05$)

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Table 10. Mean sensory scores of the three off-flavors for the three fortifications

Attributes	Light Ox	Metal Ox	Flat
No fortification	0.74^{ab}	0.40^{a}	0.62^{ab}
Vitamin E (0.06% w/w) fortification	0.64^{a}	0.49^{a}	0.46^{a}
Vitamin C (0.06% w/w) fortification	0.92^{b}	0.91^{b}	0.80^{b}

$^{a, b, c}$ within the same row, significant differences exist when means do not share the same letter ($p<0.05$)

Based on the sensory evaluation results, the 25% DDGS diet seemed to induce oxidization in milk compared to the 0% DDGS control and 10% DDGS diets ($p < 0.05$); however, the numerical value of sensory scores were very low (less than 1.5 on a 15 cm line scale).

The higher oxidized off flavor score for milk from the 25% DDGS diet could be because the fatty acid profile changed in milk from DDGS diets. However, DDGS diets (10% and 25%) had increased content of unsaturated fatty acids ($C_{18:1}$ and $C_{18:2}$); yet, the increase of unsaturated fatty acids in 10% DDGS diet milk did not contribute to a higher oxidized flavor score. Such an increase in unsaturation could have decreased the oxidative stability of the 25% DDGS diet milk. Various studies have reported milk with increased unsaturated fatty acids ($C_{18:2}$ and $C_{18:3}$) was more susceptible to oxidation (Charmley and Nicholson, 1994; Havemose et al., 2006). One study found milk with SOF had higher proportion of long-chain unsaturated fatty acids ($C_{18:1}$ and $C_{18:2}$) (Grandelli et al., 1998). Hedegaard et al. (2006) attributed the oxidative stability change in milk to the pro-oxidative effect of unsaturated fatty acids (Hedegaard et al., 2006). An increase in unsaturated fatty acids (especially all the C_{18} ones) through dietary manipulation has been reported to cause a decrease in oxidative stability in milk, and the stability was improved by supplementation of vitamin E (Focant, 1998).

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In this study, no differences in oxidized off-flavors were observed between no fortification (control) and vitamin E fortification milks. Vitamin C fortification contributed to significantly higher off-flavor scores, particularly oxidized flavors in milk (however it has to be noted again, all the scores were very low). Vitamin C seemed to decrease the oxidative stability of milk. It has been documented that under certain conditions vitamin C can act as a pro-oxidant by regenerating the perferryl radical at initiation step of lipid oxidation (Lindmark-Månsson and Åkesson, 2000). Haase and Dunkley (1969) reported that vitamin C has pro-oxidant property; it was able to catalyze the oxidation of linoleic acid (Haase and Dunkley, 1969). Additionally, vitamin C fortification in milk at 0.05% concentration has been reported to have a negative sensory impact on milk flavor (Ardt et al., 2005). Kim (2012) reported that ascorbic acid (vitamin C) showed pro-oxidant properties at about 0.02% concentration in oil-in-water emulsions, such as milk, while tocopherol had strong antioxidant capacity. Barrefors et al. (1995) observed higher $C_{18:2}$ and $C_{18:3}$ contents, lower tocopherol contents, and higher vitamin C content in the milk with SOF (Barrefors et al., 1995). Even though Havemose et al. (2006) suggested the pro-oxidative effect of unsaturated fatty acids might be more important than the antioxidative effect of tocopherol on milk fat oxidation (Havemose et al., 2006), it has been previously reported that increased tocopherol content (about 50 μg/g of milk fat) in milk improved the resistance of milk fat to oxidation (Charmley and Nicholson, 1993; Focant et al., 1998). Nicholson and St-Laurent (1991) also reported that oxidized flavor was improved in milk by increased tocopherol content in the milk. However, another study reported that an increase in milk tocopherol by 20% was ineffective in controlling oxidized flavor in milk (Charmley and

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Nicholson, 1994). Additionally, the fortification of 0.05% vitamin E in milk was not able to limit oxidized flavor (Ardt, 2005).

Milk from collection day 14 had higher off-flavors (cooked, feed, foreign, and light oxidized) than the other two collection days. The off-flavor scores got lower from collection day 14 to day 21 then to day 28 as the experiment period proceeded. Such trend may suggest that cows and their milk continued to transition during the feeding period between diets. Milk fat might have been less stable early, when the cows were switched to a new DDGS diet, however when the cows got used to the new diets, milk fat may have become more stable. Although a wash-out period of a week was allowed between diets, perhaps the transition to a new diet took longer. Additional research would be required to test this hypothesis. The inconsistency of DDGS produced within and between ethanol plants is a frequent concern for dairy producers and nutrition consultants. The variations in fat, protein, and phosphorus content make it difficult to accurately formulate cow diets (Schingoethe et al., 2009), which increase the challenge of providing consistent feed to cows. Such inconsistency could contribute to the potential of SOF development in milk, but additional research would be required to test this hypothesis.

Although significantly higher off-flavor scores were noted for the 25% DDGS diet, collection day 14, and vitamin C fortification, all of the off-flavor sensory scores were lower than 1.5 on a 15 cm line scale. So practically speaking, no real apparent oxidized off-flavors were defected in any of the milk samples and DDGS may not have contributed to a practical decrease in milk oxidative stability.

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<u>**Chemical Analysis**</u>

PV and FFA content of each milk sample from the sensory evaluation were collected from SafTest™ system and are reported in Appendix G1-G6. The PV for all milk samples were lower than 0.25 meq peroxides/kg, which was even lower than the 0.7 meq/kg PV reported in in a previous study (Let et al., 2005) for their control no-oxidation milk. The FFA contents were lower than 0.2% (g of oleic acid per 100 g of sample). All PVs and almost all FFA contents were below the detection limit. They were lower than the lowest calibrator concentration of the measurement standard curves. Low amounts of FFA were detected in several milk samples from experiment period 1, but none of these results were reproduced. Their duplicates were all below the detection concentration. High correlation between levels of primary lipid oxidation product, peroxides and the oxidized flavor in sensory analysis has been reported (Hedegaard et al., 2006). In the current study, the chemical analysis results support the sensory evaluation: no oxidation was observed. Let et al. (2005) reported that the sensory analysis was able to distinguish small differences in oxidized flavor in milk that were not detectable by chemical analysis of PV, which made it even more clear that no apparent milk oxidation happened in any of the samples in this study. Thiobarbituric acid reactive substances (TBARS) assay, measuring secondary lipid oxidation aldehyde products, was not included in the study. However, PV and sensory evaluation together are sufficient to evaluate oxidation in milk in our study. We did not detect any peroxides in any of the milk samples, which could mean two things: there was no lipid peroxides formed, hence no oxidation; or all the peroxides formed had decomposed into secondary lipid oxidation products (e.g. aldehydes and ketones). If the

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second scenario were true, the sensory panel would have detected much higher oxidized off-flavors in the milk samples. But no apparent oxidation was detected by the sensory panel in this study. So the PV and sensory results were sufficient to support our finding of no apparent oxidation in the milk samples.

In this study, the inclusion of up to 25% DDGS in the diet contributed to an increase in long-chain unsaturated fatty acids, $C_{18:1}$ and $C_{18:2}$ in milk. However, these increases did not cause any increased PV in any of the milk samples, nor was any oxidized flavor detected by the trained sensory panel. A similar situation was observed in a previous study. The higher level of oleic acid, $C_{18:1}$ did not attribute to a higher level of lipid hydroperoxides, but the elevated level of PUFA did (Havemose et al., 2006). And in another study, Liu et al. (2010) also observed decreased oxidative stability in milk with increased n-3 PUFA content in milk (Liu et al., 2010). In our study, we did not detect any differences in $C_{18:3}$ content in milk from the 3 DDGS diets (0%, 10%, and 25%), and no apparent oxidation was observed either. Havemose et al. (2004) reported that milk with lower $C_{18:1}$ and $C_{18:2}$ but higher $C_{18:3}$ had significantly higher lipid hydroperoxides content, hence oxidation (Havemose et al., 2004). Additionally, the development of SOF in milk has been related to the higher concentration of PUFA in milk fat (Timmons et al., 2001); so, it suggests that $C_{18:3}$ content could possibly be an indicator of the oxidative stability in milk. However, Timmons et al. (2001) suggested that PUFA alone is not always sufficient to evaluate the development of SOF in milk (Timmons et al., 2001). Fearon et al. (2004) did not observe a difference in oxidative stability between the treatment milk (with increased unsaturated fatty acids) and the control milk (Fearon et al., 2004).

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Thermal treatment to the milk may possibly increase the antioxidative activity in milk because of protein unfolding and exposure of thiol groups (Taylor and Richardson, 1980; Tong and others, 2000). Heat pasteurization could be one of the factors explaining why no oxidation was observed in the present study, because the milk samples were pasteurized within 4 hours after collection from the farm, which is much shorter than common industry practice.

Milk lipid oxidation is a rather complicated process. Fatty acid composition and antioxidants are only two aspects of the many factors that could influence milk oxidative stability. Pro-oxidative factors could also affect to the oxidative stability of milk. Copper is naturally present in milk, but the concentration various among cows and diets (Dunkley et al., 1968). It is thought to be one of the most potent pro-oxidants in milk, and it has a strong effect on SOF development in milk (Bruhn et al., 1976). SOF is more likely to develop in milk with higher copper content (Timmons et al., 2001). A clear association between the development of SOF and concentration of copper and PUFA in milk has been reported by (Juhlin et al., 2010). Amounts of PUFA and copper have been positively correlated to the development of SOF in milk, whereas tocopherol showed antioxidative property, negatively correlated to the SOF in milk (Bruhn et al., 1976; Juhlin et al., 2010). Lipid oxidation in milk is affected by a complex interplay of the pro-oxidants and antioxidants (Lindmark-Månsson and Åkesson, 2000). Researchers have indicated that the balance between pro-oxidants and antioxidants is a critical factor for the oxidative stability of milk (Stapefledt et al., 1999; Morales et al., 2000). Grandelli et al. (1998) related SOF in milk with the ratios between antioxidants and polyunsaturated fatty acids (PUFA). They observed milk samples without

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SOF tended to have higher antioxidant/PUFA ratios than the ones with SOF (Grandelli et al., 1998). A number of other factors have also shown influences on the oxidative stability of milk. Genetics of cows has also been mentioned as an influence on the occurrence of SOF in milk (Juhlin et al., 2010). So no single factor can be solely responsible for the development of SOF in milk.

Based on our study, DDGS alone is not responsible for SOF in milk. In the present study, carefully balanced diets were provided to the cows consistently throughout the study, with no sudden shifts (because of the washout period built into the study). Every step during milk collection and processing was carried out with extra care that was beyond the common practice. All the equipment and supplies were clean and sanitized very carefully, immediately before collection of our specific cows, to ensure milk cleanliness. Milk was handled with great care (cleanliness, minimized agitation, etc.) and pasteurization was applied to raw milk within 3 hours of collection. During transportation and storage, all milk samples were shielded from light. All these aspects could play a role in the absence of SOF in the milk in our study.

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CHAPTER 4: CONCLUSION

Feeding DDGS to cows, at 25% substitution of the dry matter, significantly decreased the milk fat content and increased the protein and SNF contents in milk. Milk fatty acid profile was also altered by DDGS inclusion. Medium-chain fatty acids decreased and long-chain unsaturated fatty acids (oleic acids and linoleic acids) increased in milk from cow fed DDGS diets. A trained sensory panel did not detect practically meaningful oxidized flavors in any of the milk samples. Chemical analysis, peroxide value and FFA content, supported the sensory results. Because no apparent oxidation was detected in milk from cows fed DDGS, the explanation for spontaneous oxidation and SOF is still unclear and DDGS cannot be solely blamed for the development of SOF in milk. No single factor is accountable for the development of SOF, but rather a combination of various factors. Resolving the problem of spontaneous oxidation in milk could be very difficult and time-consuming, because a number of factors and conditions or combinations of them can lead to the spontaneous oxidation at various stage of the milk production process.

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CHAPTER 5: FUTURE RESEARCH SUGGESTIONS

All the milk samples were blocked from light; hence light-induced oxidation in milk was eliminated from the study. However, there is the possibility of light being a contributing factor to SOF in milk. To test such a hypothesis, future research could compare light-blocked milk with light-exposed milk.

The focus of the current study was on milk lipid oxidation. Milk protein oxidation could also contribute to off-flavors in milk because of the oxidation of specific amino acids (Kim and Morr, 1996). Protein oxidation can be initiated by several factors, such as the lactoperoxidase system (Østdal et al., 2000) and photo-oxidation (Dimick, 1976). Studies have also shown antioxidative properties from milk proteins: caseins were able to inhibit lipid peroxidation by autoxidizing iron (thus inhibit iron-induced lipid peroxidation) and inhibiting formation of oxygen radicals (Cervato et al., 1999); the iron-protein complexes (1 mmol/L ferrous ion plus 10 mg/L protein) with sodium caseinate, whey protein isolate, and milk protein concentrate were able to significantly reduce iron induced linoleic acid oxidation in a oil-water emulsion model (Sugiarto et al., 2010); high molecular weight (HMW) fraction of whey protein from pasteurized milk was found to inhibit TBARS and lipid peroxides formation in a salmon oil-in-water emulsion, and its antioxidant activity increased with increased concentration (700 μg/ml to 9800 μg/ml) of HMW fraction of whey protein (Tong et al., 2000). However, Havemose et al. (2004) indicated that the formation of dityrosine, a marker for light-induced oxidation products of protein in milk, was independent of both the lipid oxidation and of the higher degree of unsaturation in milk (Havemose et al., 2004).

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All of the milk in this study was whole milk, straight from the cow, without standardizing milk fat and protein contents. Future studies could standardize the fat and/or protein content in milk to better evaluate the effect of protein and protei-lipid interaction on milk oxidative stability.

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APPENDIX A. DIET COMPOSITION IN THE THREE EXPERIMENT PERIODS

Dry Basis	Period 1		Period 2		Period 3	
	TMR	TMR + 10% DDGS	TMR + 10% DDGS	TMR + 25% DDGS	TMR	25% DDGS + TMR
Crude Protein	17.41%	16.08%	17.60%	17.37%	18.27%	19.58%
A D F	19.63%	21.56%	20.51%	21.13%	22.91%	21.24%
aN D F	30.48%	33.17%	32.18%	33.35%	34.59%	34.44%
Lignin (sulfuric acid)	3.17%	3.51%	3.32%	3.44%	3.60%	3.31%
Lignin % of NDF	10.41%	10.59%	10.32%	10.32%	10.41%	9.62%
AD-ICP % of CP	6.32%	7.65%	6.20%	7.14%	6.08%	6.03%
ADP-ICP % of DM	1.10%	1.23%	1.09%	1.24%	1.11%	1.18%
ND-ICP % of CP est	16.00%	16.00%	16.00%	16.00%	16.00%	16.00%
ND-ICP % of DM est	2.79%	2.57%	2.82%	2.78%	2.92%	3.13%
Protein Sol. % Of CP	31.30%	30.29%	30.40%	26.31%	31.03%	26.10%
Starch	28.03%	24.24%	25.05%	21.62%	23.17%	17.79%
Fat (EE)	4.47%	5.59%	5.42%	7.31%	4.63%	7.02%
Ash	6.94%	7.17%	6.58%	6.14%	6.96%	6.75%
Calcium	1.04%	0.56%	0.68%	0.59%	0.95%	0.67%
Phosphorous	0.38%	0.47%	0.49%	0.57%	0.39%	0.53%
Magnesium	0.33%	0.28%	0.27%	0.27%	0.34%	0.30%
Potassium	1.29%	1.38%	1.29%	1.27%	1.32%	1.22%
Sulfur	0.22%	0.26%	0.24%	0.31%	0.21%	0.33%
Sugar (ESC)	4.00%	4.31%	2.90%	2.43%	3.12%	2.69%
Manganese	65 ppm	38 ppm	35 ppm	31 ppm	64 ppm	35 ppm
Zinc	84 ppm	58 ppm	61 ppm	75 ppm	98 ppm	70 ppm
Copper	22 ppm	16 ppm	15 ppm	17 ppm	24 ppm	19 ppm
Iron	298 ppm	226 ppm	198 ppm	185 ppm	257 ppm	229 ppm
Sodium	0.26%	0.13%	0.16%	0.17%	0.26%	0.15%
Molybdenum	0.88 ppm	0.92 ppm	0.99 ppm	0.85%	0.73%	0.81 ppm

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APPENDIX B. SENSORY EVALUATION CONSENT FORM

Consent to Participate in Milk taste Panel

You are being asked to take part in a research study carried out by *research team of Dr. Stephanie Clark, Food science and Human Nutrition.* This form explains the research study and your part in it if you decide to join the study. Please read the form carefully, taking as much time as you need. Ask the research staff to explain anything you don't understand. You can decide not to join the study. If you join the study, you can change your mind later or quit at any time. There will be no penalty or loss of services or benefits if you decide to not take part in the study or quit later. This study has been approved for human subject participation by the Iowa State University Institutional Review Board.

This research study is being done to *understand the effect of distillers grain feeding upon milk quality and shelf life.* To take part in this study, you must consume milk at least weekly and have ***no aversions to dairy products.*** Taking part in the study will take approximately 60 minutes twice a week (Tuesdays and Thursdays), between June 28, 2011 and November 10, 2011 (with appropriate breaks given to accommodate panelists' schedules). If you take part in the study, you will be asked to provide us with a schedule of your availability and to attend all training and tasting sessions. If you miss more than two training or tasting sessions, you will be asked to discontinue the study due to our need for complete data collection. You may elect to drop out of the study at any time. Without any negative feelings, but ill forfeit compensation for sessions not completed. At the end of the study, you will be compensated with $5 for each completed training or tasting session, for a total of approximately $175.

There is no direct benefit to you from being in this study except the financial compensation for completed sessions. The milk samples will be prepared using sanitary procedures and are safe for your consumption, however you will not be asked to swallow the samples. The potential risks from taking part in this study are potential *dislike for the flavor of the products, stress in using the sensory ballots. To minimize these issues, you do not have to swallow any sample; nobody will watch you answer questions or pressure you to finish.*

The data for this study will be kept confidential to the extent allowed by federal and stat law. No published results will identify you, and your name will not be associated with the findings.

If you have questions about this study or the information in this form, please contact the principal investigator of the project, Dr. Stephanie Clark, at 515-294-7346 or milkmade@iastate.edu.

Your signature on this form means that:

- You understand the information given to you in this form
- You have been able to ask the researcher questions and state any concerns
- The researcher has responded to your questions and concerns
- You believe you understand the research study and the potential benefits and risks involved.

Statement of Content

I give my voluntary consent to take part in this study. Upon request, I will be given a copy of this consent document for my records.

______________________________ ______________________

Signature of Participant Date

____

Printed Name of Participant

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APPENDIX C. SENSORY EVALUATION TRAINING BALLOT

Panelist Number_______ Sample #__________

EVALUATION OF MILK FLAVOR

Indicate the level of aroma/flavor noted by marking a perpendicular line on the scale.

Cooked

______|____|______

weak strong

Feed

______|____|______

weak strong

Foreign (describe:____)

______|____|______

weak strong

Light Oxidized

______|____|______

weak strong

Flat

______|____|______

weak strong

Metal Oxidized

______|____|______

weak strong

Bitter

______|____|______

weak strong

Comments:

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APPENDIX D. PREPARATION FOR SPECIFIC OFF-FLAVORS IN MILK FOR SENSORY TRAINING SESSIONS

Off-flavor attribute	Preparation
Bitter	(Definite) Prepare 0.5% quinine (Sigma-Aldrich, St. Louis, MO) solution with deionized water. Add 1/2 teaspoon of the quinine solution to 20 oz. of no defect whole milk, paperboard/light-blocking container (Anderson Erickson Dairy, Inc., Des Moines, IA).
Cooked	(Definite) Organic whole milk, Ultrapasteurized, paperboard container (Organic Valley, La Farge, WI).
Feed	(Pronounced) Boil about 1.5 L of water. Put a handful of dried alfalfa hay into a heatproof bowl. Pour the boiling water into the bowl with the alfalfa hay. Let it steep for about 5 minutes. Filter the tea through a coffee filter. Add 3 teaspoons of the alfalfa tea to 20 oz. of no defect whole milk, paperboard/light-blocking container.
Flat	(Definite) Store-bought 1% milk, paperboard/light-blocking container (Anderson Erickson Dairy, Inc., Des Moines, IA).
Foreign (chlorine/sanitizer)	(Definite) Prepare a sanitizer solution by adding 2 tablespoons of bleach (Clorox® Bleach, Oakland, CA) to a gallon of tap water. Add 2 teaspoons of the sanitizer solution to 20 oz. of no defect whole milk, paperboard/light-blocking container.
Light oxidized	(Pronounced) Store-bought whole milk in **clear** plastic or glass container. (Anderson Erickson Dairy, Inc., Des Moines, IA). (Definite) Store-bought whole milk in **translucent** plastic containers (Anderson Erickson Dairy, Inc., Des Moines, IA). (Slight) 1:2 dilution of "definite" milk with no defect whole milk.
Metal oxidized	(Pronounced) Prepare 0.25% cupric sulfate (Sigma-Aldrich, St. Louis, MO) solution with deionized water. Add 1/2 teaspoon of solution to 20 oz. of no defect milk; let it stand refrigerated for 90 min prior to training. (Definite) 1:2 dilution of "pronounced" milk with no defect whole milk. (Slight) 1:4 dilution of "pronounced" milk with no defect whole milk.

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APPENDIX E1. MILK FAT CONTENTS FOR EACH INDIVIDUAL COW IN THE NINE MEASUREMENTS

Fat	0% DDGS			10% DDGS			25% DDGS		
Cow #	14	21	28	14	21	28	14	21	28
6763	3.35	3.64	3.42	n/a	2.28	2.59	2.78	2.88	2.54
7965	3.68	3.44	3.14	n/a	2.86	2.76	2.62	2.45	2.86
6742	3.52	3.59	3.49	n/a	2.88	2.66	2.08	2.72	1.70
7065	2.98	1.95	2.32	n/a	2.26	2.15	2.04	2.08	2.73
6684	3.46	3.64	3.62	n/a	3.90	2.14	2.64	3.04	2.36
7117	3.81	3.20	3.82	n/a	1.73	1.56	1.82	2.55	2.26
7583	3.72	3.66	3.89	n/a	2.88	2.79	2.90	2.30	2.80
6783	4.24	3.69	4.02	n/a	2.01	3.07	2.14	2.37	2.38
6716	3.10	3.15	4.68	n/a	2.08	2.06	2.44	2.30	2.70
7503	3.44	3.40	3.40	n/a	4.57	3.90	2.71	3.48	2.76
8005	n/a	3.30	2.26	1.82	2.41	2.16	2.20	2.48	2.65
7986	n/a	2.56	2.57	2.84	2.66	2.50	3.27	2.72	2.6
6753	n/a	2.51	2.32	2.48	3.48	3.52	3.56	3.93	3.06
6384	n/a	3.82	2.91	2.18	1.84	3.05	2.25	2.51	2.5
6570	n/a	2.94	3.28	3.28	2.54	2.62	3.22	2.79	2.49
6525	n/a	1.84	2.53	2.74	3.10	3.27	2.40	2.69	3.62
7385	n/a	2.56	2.49	2.49	2.27	2.63	2.98	3.56	3.40
6499	n/a	4.28	4.12	5.00	4.62	3.66	2.76	3.40	2.64
7954	n/a	3.49	3.50	2.35	2.23	2.46	2.68	4.07	3.68
6657	n/a	3.02	2.66	2.92	n/a	n/a	3.34	3.66	2.73
7571	n/a	n/a	n/a	2.96	2.42	2.92	2.78	2.90	2.15
7049	n/a	n/a	n/a	2.56	2.34	1.80	2.16	2.45	1.82
7627	n/a	n/a	n/a	n/a	n/a	n/a	2.62	2.64	2.52
7378	n/a	n/a	n/a	n/a	n/a	n/a	3.37	2.80	3.08
7288	5.34	3.22	2.71	n/a	n/a	n/a	n/a	n/a	n/a

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APPENDIX E2. MILK PROTEIN CONTENTS FOR EACH INDIVIDUAL COW IN THE NINE MEASUREMENTS

Protein	0% DDGS			10% DDGS			25% DDGS		
Cow #	14	21	28	14	21	28	14	21	28
6763	3.98	3.90	3.96	n/a	4.02	3.88	4.11	4.06	4.11
7965	3.90	3.84	3.86	n/a	3.80	3.86	4.02	4.06	4.00
6742	3.74	3.71	3.76	n/a	3.68	3.69	3.72	3.90	3.88
7065	3.60	3.68	3.71	n/a	3.66	3.82	3.88	3.82	3.93
6684	3.80	3.79	3.80	n/a	3.70	3.81	3.92	3.9	3.94
7117	3.88	3.74	3.86	n/a	3.62	3.77	3.88	3.78	3.81
7583	3.68	3.72	3.62	n/a	3.79	3.86	3.90	3.90	3.86
6783	3.57	3.68	3.73	n/a	3.70	3.76	3.92	3.87	3.92
6716	3.80	3.72	3.72	n/a	3.94	3.91	4.10	3.94	4.01
7503	3.66	3.65	3.63	n/a	3.50	3.54	3.71	3.68	3.73
8005	n/a	3.81	3.84	4.00	3.75	4.08	3.99	3.96	3.92
7986	n/a	3.68	3.69	3.75	3.87	3.93	3.93	3.96	4.02
6753	n/a	3.49	3.42	3.52	3.50	3.74	3.85	3.57	3.60
6384	n/a	3.23	3.66	3.72	4.00	3.82	3.92	3.87	3.84
6570	n/a	3.88	3.82	3.92	3.88	3.97	4.02	3.97	3.94
6525	n/a	3.64	3.62	3.66	3.62	3.72	3.77	3.59	3.58
7385	n/a	3.70	3.78	3.90	3.90	4.00	3.92	3.70	3.78
6499	n/a	3.70	3.68	3.77	3.73	3.81	3.81	3.74	3.94
7954	n/a	3.69	3.64	3.77	3.74	3.77	3.76	3.64	3.72
6657	n/a	3.58	3.60	3.64	n/a	n/a	3.69	3.66	3.73
7571	n/a	n/a	n/a	3.78	3.82	3.93	3.86	3.9	4.02
7049	n/a	n/a	n/a	3.54	3.63	3.76	3.66	3.64	3.61
7627	n/a	n/a	n/a	n/a	n/a	n/a	3.94	3.72	4.00
7378	n/a	n/a	n/a	n/a	n/a	n/a	3.7	3.66	3.72
7288	3.54	3.60	3.72	n/a	n/a	n/a	n/a	n/a	n/a

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APPENDIX E3. MILK SNF CONTENTS FOR EACH INDIVIDUAL COW IN THE NINE MEASUREMENTS

SNF	0% DDGS			10% DDGS			25% DDGS		
Cow #	14	21	28	14	21	28	14	21	28
6763	10.50	10.40	10.60	n/a	10.70	10.40	10.90	10.80	10.90
7965	10.40	10.20	10.20	n/a	10.10	10.30	10.70	10.80	10.60
6742	9.94	9.86	10.00	n/a	9.81	9.82	9.92	10.40	10.30
7065	9.58	9.96	9.90	n/a	9.75	10.20	10.30	10.20	10.50
6684	10.00	10.10	10.10	n/a	9.81	10.20	10.40	10.40	10.50
7117	10.30	9.93	10.3	n/a	9.68	10.10	10.40	10.10	10.20
7583	9.78	9.88	9.60	n/a	10.10	10.30	10.40	10.40	10.30
6783	9.48	9.76	9.90	n/a	9.98	10.00	10.50	10.30	10.40
6716	10.10	9.90	9.86	n/a	10.50	10.40	10.90	10.50	10.70
7503	9.72	9.69	9.64	n/a	9.27	9.39	9.88	9.79	9.94
8005	n/a	10.10	10.20	10.70	10.00	10.90	10.60	10.60	10.40
7986	n/a	9.82	9.84	9.99	10.30	10.40	10.40	10.50	10.7
6753	n/a	9.28	9.11	9.38	9.29	9.94	10.20	9.47	9.63
6384	n/a	9.55	9.72	9.92	10.70	10.20	10.40	10.30	10.20
6570	n/a	10.3	10.20	10.40	10.40	10.60	10.70	10.60	10.5
6525	n/a	9.73	9.62	9.76	9.62	9.90	10.00	9.56	9.50
7385	n/a	9.84	10.10	10.4	10.40	10.70	10.40	9.82	10.10
6499	n/a	9.81	9.78	9.98	9.88	10.10	10.10	9.92	10.5
7954	n/a	9.81	9.66	10.10	9.98	10.00	10.00	9.66	9.89
6657	n/a	9.53	9.60	9.70	n/a	n/a	9.81	9.74	9.94
7571	n/a	n/a	n/a	10	10.2	10.50	10.20	10.40	10.70
7049	n/a	n/a	n/a	9.42	9.68	10.00	9.75	9.62	9.63
7627	n/a	n/a	n/a	n/a	n/a	n/a	10.5	9.91	10.60
7378	n/a	n/a	n/a	n/a	n/a	n/a	9.84	9.76	9.90
7288	9.37	9.59	9.89	n/a	n/a	n/a	n/a	n/a	n/a

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APPENDIX F1. SENSORY SCORES* FOR CONTROL DIET TREATMENT MILKS

	No vitamin			Vitamin E			Vitamin C		
Collection day 14	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	1.89	1.38	0.37	1.00	0.27	0.70	0.70	0.78	0.65
Feed	0.33	0.38	0.13	0.41	0.26	0.40	0.73	0.44	0.52
Foreign	0.98	0.75	0.75	2.30	0.98	1.55	2.06	0.55	1.40
Light Oxidized	0.54	0.40	0.94	0.76	1.37	0.90	0.86	2.02	0.67
Flat	0.66	0.96	0.45	0.38	1.01	0.62	1.06	0.79	0.35
Bitter	0.09	0.09	0.11	0.08	0.02	0.50	0.22	0.01	0.46
Metal Oxidized	0.49	0.38	0.10	0.92	0.25	0.78	1.37	1.10	1.02
	No vitamin			**Vitamin E**			**Vitamin C**		
Collection day 21	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.97	0.14	0.40	0.59	0.64	0.05	1.13	0.24	0.67
Feed	0.35	0.23	0.16	0.30	0.58	0.46	0.05	0.34	0.17
Foreign	0.12	0.02	0.52	0.43	0.32	0.99	0.56	0.25	1.06
Light Oxidized	0.70	0.68	0.96	0.52	0.92	0.23	0.37	0.30	0.89
Flat	0.13	1.33	0.27	0.69	0.91	0.70	0.96	0.10	0.42
Bitter	0.19	0.02	0.08	0.20	0.03	1.31	0.04	0.16	0.15
Metal Oxidized	0.53	0.10	0.24	1.20	0.04	0.84	0.89	0.52	0.45
	No vitamin			**Vitamin E**			**Vitamin C**		
Collection day 28	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.72	0.32	0.22	0.06	0.23	0.17	0.36	0.25	0.24
Feed	0.17	0.47	0.34	0.02	0.16	0.16	0.25	0.20	0.13
Foreign	0.52	0.11	0.57	0.44	0.52	0.65	0.59	0.82	1.16
Light Oxidized	0.51	0.70	0.90	0.81	0.48	0.63	0.53	0.95	1.30
Flat	0.20	0.24	0.07	1.00	0.88	0.38	0.58	0.10	0.25
Bitter	0.17	0.34	0.02	0.05	0.19	0.39	0.03	0.03	0.35
Metal Oxidized	0.46	0.46	0.04	0.22	0.13	0.85	0.27	0.54	0.34

*Scores are the average from the 10 panelists; scores were on a 15 cm scale.

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APPENDIX F2. SENSORY SCORES* FOR 10% DDGS DIET TREATMENT MILKS

	No vitamin			Vitamin E			Vitamin C		
Collection day 14	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.65	0.74	1.03	0.47	0.81	0.42	1.19	0.77	0.62
Feed	0.71	0.23	0.34	0.09	0.16	0.86	0.76	0.39	0.21
Foreign	1.14	0.81	0.50	0.69	0.71	0.61	0.69	0.51	0.47
Light Oxidized	1.18	1.38	0.89	0.82	0.67	0.94	1.15	0.93	1.35
Flat	0.52	1.05	0.36	1.62	0.56	0.69	0.84	0.19	0.75
Bitter	0.50	0.21	0.09	0.17	0.25	0.09	0.13	0.10	0.21
Metal Oxidized	0.56	0.09	0.10	0.05	0.25	0.13	0.59	0.81	0.33

	No vitamin			Vitamin E			Vitamin C		
Collection day 21	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.39	0.63	0.30	0.57	0.26	0.58	0.35	0.36	0.24
Feed	0.15	0.17	0.20	0.47	0.16	0.43	0.26	0.05	0.24
Foreign	0.26	0.24	0.31	0.29	0.19	0.76	1.00	0.22	0.01
Light Oxidized	0.90	0.75	0.29	0.34	0.74	0.66	0.75	1.56	1.02
Flat	0.57	1.00	1.04	0.99	0.57	0.72	0.69	0.92	0.46
Bitter	0.03	0.16	0.01	0.28	0.03	0.12	0.13	0.51	0.33
Metal Oxidized	0.13	0.56	0.30	0.23	0.88	0.73	0.53	0.26	1.07

	No vitamin			Vitamin E			Vitamin C		
Collection day 28	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.60	0.67	0.18	0.23	0.36	0.39	0.55	0.74	0.15
Feed	0.25	0.40	0.03	0.19	0.11	0.01	0.18	0.07	0.10
Foreign	0.13	0.38	0.45	0.31	1.08	0.88	0.53	0.27	0.16
Light Oxidized	0.92	0.39	1.01	0.39	0.45	0.31	0.69	0.76	0.33
Flat	0.66	0.22	0.91	0.31	0.55	1.55	0.79	0.14	0.44
Bitter	0.04	0.11	0.10	0.21	0.17	0.01	0.04	0.09	0.12
Metal Oxidized	0.64	0.21	0.70	0.59	0.61	0.27	0.55	0.72	0.34

*Scores are the average from the 10 panelists; scores were on a 15 cm scale.

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APPENDIX F3. SENSORY SCORES* FOR 25% DDGS DIET TREATMENT MILKS

	No vitamin			Vitamin E			Vitamin C		
Collection day 14	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.60	0.93	0.20	0.49	0.46	0.58	0.14	0.55	0.77
Feed	0.36	0.07	0.52	0.46	0.12	0.22	0.11	0.11	0.27
Foreign	1.90	1.58	1.28	0.83	0.82	1.32	2.11	0.77	1.68
Light Oxidized	0.54	0.92	0.72	0.67	0.33	0.70	0.67	0.75	0.76
Flat	1.53	1.01	0.32	0.27	0.28	0.51	0.90	0.31	0.35
Bitter	0.05	0.39	0.01	0.31	0.14	0.12	0.06	0.17	0.11
Metal Oxidized	0.72	0.57	0.70	0.46	1.02	0.32	1.18	1.68	1.39
	No vitamin			**Vitamin E**			**Vitamin C**		
Collection day 21	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.08	0.17	0.31	0.11	0.71	0.42	0.24	0.28	0.24
Feed	0.12	0.01	0.19	0.34	0.20	0.01	0.21	0.11	0.10
Foreign	1.07	0.61	0.69	0.38	0.48	0.48	1.22	0.83	0.59
Light Oxidized	0.83	0.47	1.14	0.92	0.58	0.56	1.11	1.22	0.99
Flat	0.59	0.71	0.56	0.75	0.93	0.39	0.08	0.02	0.27
Bitter	0.19	0.00	0.01	0.07	0.01	0.07	0.01	0.06	0.20
Metal Oxidized	0.58	0.09	0.35	0.97	0.19	0.53	2.05	2.04	1.45
	No vitamin			**Vitamin E**			**Vitamin C**		
Collection day 28	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7	Storage day 1	Day 3	Day 7
Cooked	0.21	0.02	0.70	0.25	0.37	0.66	0.42	0.56	0.05
Feed	0.01	0.24	0.04	0.02	0.10	0.60	0.37	0.08	0.44
Foreign	0.41	0.74	0.42	0.83	1.14	0.30	0.55	1.62	0.90
Light Oxidized	0.75	0.15	0.36	0.47	0.39	0.81	1.00	0.95	1.12
Flat	0.22	0.68	0.50	0.89	0.64	0.72	0.06	0.03	0.31
Bitter	0.06	0.09	0.07	0.07	0.02	0.05	0.10	0.03	0.65
Metal Oxidized	0.59	0.34	0.96	0.52	0.22	0.13	1.42	0.57	1.14

*Scores are the average from the 10 panelists; scores were on a 15 cm scale.

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APPENDIX G1. PEROXIDE VALUES FOR MILK SAMPLES IN EXPERIMENT PERIOD 1

Rep #	Collection day	Evaluation day	Treatment	Sample	meq of peroxides/kg)
1	14	1	CONTROL	Control	< 0.5
1	14	1	COWS	Vitamin E	< 0.5
1	14	1		Vitamin C	< 0.5
1	14	1	10% DIET	Control	< 0.5
1	14	1	COWS	Vitamin E	< 0.5
1	14	1		Vitamin C	< 0.5
1	14	3	CONTROL	Control	< 0.5
1	14	3	COWS	Vitamin E	< 0.5
1	14	3		Vitamin C	< 0.5
1	14	3	10% DIET	Control	< 0.5
1	14	3	COWS	Vitamin E	< 0.5
1	14	3		Vitamin C	< 0.5
1	14	7	CONTROL	Control	< 0.5
1	14	7	COWS	Vitamin E	< 0.5
1	14	7		Vitamin C	< 0.5
1	14	7	10% DIET	Control	< 0.5
1	14	7	COWS	Vitamin E	< 0.5
1	14	7		Vitamin C	< 0.5
1	21	1	CONTROL	Control	< 0.5
1	21	1	COWS	Vitamin E	< 0.5
1	21	1		Vitamin C	< 0.5
1	21	1	10% DIET	Control	< 0.5
1	21	1	COWS	Vitamin E	< 0.5
1	21	1		Vitamin C	< 0.5
1	21	3	CONTROL	Control	< 0.5
1	21	3	COWS	Vitamin E	< 0.5
1	21	3		Vitamin C	< 0.5
1	21	3	10% DIET	Control	< 0.5
1	21	3	COWS	Vitamin E	< 0.5
1	21	3		Vitamin C	< 0.5
1	21	7	CONTROL	Control	< 0.25
1	21	7	COWS	Vitamin E	< 0.25
1	21	7		Vitamin C	< 0.25
1	21	7	10% DIET	Control	< 0.25
1	21	7	COWS	Vitamin E	< 0.25
1	21	7		Vitamin C	< 0.25
1	28	1	CONTROL	Control	< 0.25
1	28	1	COWS	Vitamin E	< 0.25
1	28	1		Vitamin C	< 0.25
1	28	1	10% DIET	Control	< 0.25
1	28	1	COWS	Vitamin E	< 0.25
1	28	1		Vitamin C	< 0.25
1	28	3	CONTROL	Control	< 0.25
1	28	3	COWS	Vitamin E	< 0.25
1	28	3		Vitamin C	< 0.25
1	28	3	10% DIET	Control	< 0.25
1	28	3	COWS	Vitamin E	< 0.25
1	28	3		Vitamin C	< 0.25
1	28	7	CONTROL	Control	< 0.25
1	28	7	COWS	Vitamin E	< 0.25
1	28	7		Vitamin C	< 0.25
1	28	7	10% DIET	Control	< 0.25
1	28	7	COWS	Vitamin E	< 0.25
1	28	7		Vitamin C	< 0.25

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APPENDIX G2. PEROXIDE VALUES FOR MILK SAMPLES IN EXPERIMENT PERIOD 2

Rep #	Collection day	Evaluation day	Treatment	Sample	(meq of peroxides/kg)
2	14	1	10% DIET	Control	< 0.25
2	14	1	COWS	Vitamin E	< 0.25
2	14	1		Vitamin C	< 0.25
2	14	1	25% DIET	Control	< 0.25
2	14	1	COWS	Vitamin E	< 0.25
2	14	1		Vitamin C	< 0.25
2	14	3	10% DIET	Control	< 0.25
2	14	3	COWS	Vitamin E	< 0.25
2	14	3		Vitamin C	< 0.25
2	14	3	25% DIET	Control	< 0.25
2	14	3	COWS	Vitamin E	< 0.25
2	14	3		Vitamin C	< 0.25
2	14	7	10% DIET	Control	< 0.25
2	14	7	COWS	Vitamin E	< 0.25
2	14	7		Vitamin C	< 0.25
2	14	7	25% DIET	Control	< 0.25
2	14	7	COWS	Vitamin E	< 0.25
2	14	7		Vitamin C	< 0.25
2	21	1	10% DIET	Control	< 0.25
2	21	1	COWS	Vitamin E	< 0.25
2	21	1		Vitamin C	< 0.25
2	21	1	25% DIET	Control	< 0.25
2	21	1	COWS	Vitamin E	< 0.25
2	21	1		Vitamin C	< 0.25
2	21	3	10% DIET	Control	< 0.25
2	21	3	COWS	Vitamin E	< 0.25
2	21	3		Vitamin C	< 0.25
2	21	3	25% DIET	Control	< 0.25
2	21	3	COWS	Vitamin E	< 0.25
2	21	3		Vitamin C	< 0.25
2	21	7	10% DIET	Control	< 0.25
2	21	7	COWS	Vitamin E	< 0.25
2	21	7		Vitamin C	< 0.25
2	21	7	25% DIET	Control	< 0.25
2	21	7	COWS	Vitamin E	< 0.25
2	21	7		Vitamin C	< 0.25
2	28	1	10% DIET	Control	< 0.25
2	28	1	COWS	Vitamin E	< 0.25
2	28	1		Vitamin C	< 0.25
2	28	1	25% DIET	Control	< 0.25
2	28	1	COWS	Vitamin E	< 0.25
2	28	1		Vitamin C	< 0.25
2	28	3	10% DIET	Control	< 0.25
2	28	3	COWS	Vitamin E	< 0.25
2	28	3		Vitamin C	< 0.25
2	28	3	25% DIET	Control	< 0.25
2	28	3	COWS	Vitamin E	< 0.25
2	28	3		Vitamin C	< 0.25
2	28	7	10% DIET	Control	< 0.25
2	28	7	COWS	Vitamin E	< 0.25
2	28	7		Vitamin C	< 0.25
2	28	7	25% DIET	Control	< 0.25
2	28	7	COWS	Vitamin E	< 0.25
2	28	7		Vitamin C	< 0.25

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APPENDIX G3. PEROXIDE VALUES FOR MILK SAMPLES IN EXPERIMENT PERIOD 3

Rep #	Collection day	Evaluation day	Treatment	Sample	(meq of peroxides/kg)
3	14	1	10% DIET	Control	< 0.25
3	14	1	COWS	Vitamin E	< 0.25
3	14	1		Vitamin C	< 0.25
3	14	1	25% DIET	Control	< 0.25
3	14	1	COWS	Vitamin E	< 0.25
3	14	1		Vitamin C	< 0.25
3	14	3	10% DIET	Control	< 0.25
3	14	3	COWS	Vitamin E	< 0.25
3	14	3		Vitamin C	< 0.25
3	14	3	25% DIET	Control	< 0.25
3	14	3	COWS	Vitamin E	< 0.25
3	14	3		Vitamin C	< 0.25
3	14	7	10% DIET	Control	< 0.25
3	14	7	COWS	Vitamin E	< 0.25
3	14	7		Vitamin C	< 0.25
3	14	7	25% DIET	Control	< 0.25
3	14	7	COWS	Vitamin E	< 0.25
3	14	7		Vitamin C	< 0.25
3	21	1	10% DIET	Control	< 0.25
3	21	1	COWS	Vitamin E	< 0.25
3	21	1		Vitamin C	< 0.25
3	21	1	25% DIET	Control	< 0.25
3	21	1	COWS	Vitamin E	< 0.25
3	21	1		Vitamin C	< 0.25
3	21	3	10% DIET	Control	< 0.25
3	21	3	COWS	Vitamin E	< 0.25
3	21	3		Vitamin C	< 0.25
3	21	3	25% DIET	Control	< 0.25
3	21	3	COWS	Vitamin E	< 0.25
3	21	3		Vitamin C	< 0.25
3	21	7	10% DIET	Control	< 0.25
3	21	7	COWS	Vitamin E	< 0.25
3	21	7		Vitamin C	< 0.25
3	21	7	25% DIET	Control	< 0.25
3	21	7	COWS	Vitamin E	< 0.25
3	21	7		Vitamin C	< 0.25
3	28	1	10% DIET	Control	< 0.25
3	28	1	COWS	Vitamin E	< 0.25
3	28	1		Vitamin C	< 0.25
3	28	1	25% DIET	Control	< 0.25
3	28	1	COWS	Vitamin E	< 0.25
3	28	1		Vitamin C	< 0.25
3	28	3	10% DIET	Control	< 0.25
3	28	3	COWS	Vitamin E	< 0.25
3	28	3		Vitamin C	< 0.25
3	28	3	25% DIET	Control	< 0.25
3	28	3	COWS	Vitamin E	< 0.25
3	28	3		Vitamin C	< 0.25
3	28	7	10% DIET	Control	< 0.25
3	28	7	COWS	Vitamin E	< 0.25
3	28	7		Vitamin C	< 0.25
3	28	7	25% DIET	Control	< 0.25
3	28	7	COWS	Vitamin E	< 0.25
3	28	7		Vitamin C	< 0.25

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APPENDIX G4. FFA CONTENTS FOR MILK SAMPLES IN EXPERIMENT PERIOD 1

Rep #	Collection day	Evaluation day	Treatment	Sample	SafTest (% Oleic Acid)
1	14	1	CONTROL	Control	< 0.4%
1	14	1	COWS	Vitamin E	< 0.4%
1	14	1		Vitamin C	< 0.4%
1	14	1	10% DIET	Control	< 0.4%
1	14	1	COWS	Vitamin E	< 0.4%
1	14	1		Vitamin C	< 0.4%
1	14	3	CONTROL	Control	< 0.4%
1	14	3	COWS	Vitamin E	< 0.4%
1	14	3		Vitamin C	< 0.4%
1	14	3	10% DIET	Control	< 0.4%
1	14	3	COWS	Vitamin E	< 0.4%
1	14	3		Vitamin C	< 0.4%
1	14	7	CONTROL	Control	< 0.4%
1	14	7	COWS	Vitamin E	< 0.4%
1	14	7		Vitamin C	0.81%
1	14	7	10% DIET	Control	< 0.4%
1	14	7	COWS	Vitamin E	0.77%
1	14	7		Vitamin C	< 0.4%
1	21	1	CONTROL	Control	0.77%
1	21	1	COWS	Vitamin E	0.60%
1	21	1		Vitamin C	0.88%
1	21	1	10% DIET	Control	1.01%
1	21	1	COWS	Vitamin E	1.03%
1	21	1		Vitamin C	1.05%
1	21	3	CONTROL	Control	0.83%
1	21	3	COWS	Vitamin E	< 0.4%
1	21	3		Vitamin C	0.70%
1	21	3	10% DIET	Control	< 0.4%
1	21	3	COWS	Vitamin E	0.72%
1	21	3		Vitamin C	0.70%
1	21	7	CONTROL	Control	< 0.2%
1	21	7	COWS	Vitamin E	< 0.2%
1	21	7		Vitamin C	< 0.2%
1	21	7	10% DIET	Control	< 0.2%
1	21	7	COWS	Vitamin E	< 0.2%
1	21	7		Vitamin C	< 0.2%
1	28	1	CONTROL	Control	< 0.2%
1	28	1	COWS	Vitamin E	< 0.2%
1	28	1		Vitamin C	< 0.2%
1	28	1	10% DIET	Control	< 0.2%
1	28	1	COWS	Vitamin E	< 0.2%
1	28	1		Vitamin C	< 0.2%
1	28	3	CONTROL	Control	< 0.2%
1	28	3	COWS	Vitamin E	< 0.2%
1	28	3		Vitamin C	< 0.2%
1	28	3	10% DIET	Control	< 0.2%
1	28	3	COWS	Vitamin E	< 0.2%
1	28	3		Vitamin C	< 0.2%
1	28	7	CONTROL	Control	< 0.2%
1	28	7	COWS	Vitamin E	< 0.2%
1	28	7		Vitamin C	< 0.2%
1	28	7	10% DIET	Control	< 0.2%
1	28	7	COWS	Vitamin E	< 0.2%
1	28	7		Vitamin C	< 0.2%

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APPENDIX G5. FFA CONTENTS FOR MILK SAMPLES IN EXPERIMENT PERIOD 2

Rep #	Collection day	Evaluation day	Treatment	Sample	SafTest (% Oleic Acid)
2	14	1	CONTROL	Control	< 0.2%
2	14	1	COWS	Vitamin E	< 0.2%
2	14	1		Vitamin C	< 0.2%
2	14	1	10% DIET	Control	< 0.2%
2	14	1	COWS	Vitamin E	< 0.2%
2	14	1		Vitamin C	< 0.2%
2	14	3	CONTROL	Control	< 0.2%
2	14	3	COWS	Vitamin E	< 0.2%
2	14	3		Vitamin C	< 0.2%
2	14	3	10% DIET	Control	< 0.2%
2	14	3	COWS	Vitamin E	< 0.2%
2	14	3		Vitamin C	< 0.2%
2	14	7	CONTROL	Control	< 0.2%
2	14	7	COWS	Vitamin E	< 0.2%
2	14	7		Vitamin C	< 0.2%
2	14	7	10% DIET	Control	< 0.2%
2	14	7	COWS	Vitamin E	< 0.2%
2	14	7		Vitamin C	< 0.2%
2	21	1	CONTROL	Control	< 0.2%
2	21	1	COWS	Vitamin E	< 0.2%
2	21	1		Vitamin C	< 0.2%
2	21	1	10% DIET	Control	< 0.2%
2	21	1	COWS	Vitamin E	< 0.2%
2	21	1		Vitamin C	< 0.2%
2	21	3	CONTROL	Control	< 0.2%
2	21	3	COWS	Vitamin E	< 0.2%
2	21	3		Vitamin C	< 0.2%
2	21	3	10% DIET	Control	< 0.2%
2	21	3	COWS	Vitamin E	< 0.2%
2	21	3		Vitamin C	< 0.2%
2	21	7	CONTROL	Control	< 0.2%
2	21	7	COWS	Vitamin E	< 0.2%
2	21	7		Vitamin C	< 0.2%
2	21	7	10% DIET	Control	< 0.2%
2	21	7	COWS	Vitamin E	< 0.2%
2	21	7		Vitamin C	< 0.2%
2	28	1	CONTROL	Control	< 0.2%
2	28	1	COWS	Vitamin E	< 0.2%
2	28	1		Vitamin C	< 0.2%
2	28	1	10% DIET	Control	< 0.2%
2	28	1	COWS	Vitamin E	< 0.2%
2	28	1		Vitamin C	< 0.2%
2	28	3	CONTROL	Control	< 0.2%
2	28	3	COWS	Vitamin E	< 0.2%
2	28	3		Vitamin C	< 0.2%
2	28	3	10% DIET	Control	< 0.2%
2	28	3	COWS	Vitamin E	< 0.2%
2	28	3		Vitamin C	< 0.2%
2	28	7	CONTROL	Control	< 0.2%
2	28	7	COWS	Vitamin E	< 0.2%
2	28	7		Vitamin C	< 0.2%
2	28	7	10% DIET	Control	< 0.2%
2	28	7	COWS	Vitamin E	< 0.2%
2	28	7		Vitamin C	< 0.2%

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APPENDIX G6. FFA CONTENTS FOR MILK SAMPLES IN EXPERIMENT PERIOD 3

Rep #	Collection day	Evaluation day	Treatment	Sample	SafTest (% Oleic Acid)
3	14	1	CONTROL	Control	< 0.2%
3	14	1	COWS	Vitamin E	< 0.2%
3	14	1		Vitamin C	< 0.2%
3	14	1	10% DIET	Control	< 0.2%
3	14	1	COWS	Vitamin E	< 0.2%
3	14	1		Vitamin C	< 0.2%
3	14	3	CONTROL	Control	< 0.2%
3	14	3	COWS	Vitamin E	< 0.2%
3	14	3		Vitamin C	< 0.2%
3	14	3	10% DIET	Control	< 0.2%
3	14	3	COWS	Vitamin E	< 0.2%
3	14	3		Vitamin C	< 0.2%
3	14	7	CONTROL	Control	< 0.2%
3	14	7	COWS	Vitamin E	< 0.2%
3	14	7		Vitamin C	< 0.2%
3	14	7	10% DIET	Control	< 0.2%
3	14	7	COWS	Vitamin E	< 0.2%
3	14	7		Vitamin C	< 0.2%
3	21	1	CONTROL	Control	< 0.2%
3	21	1	COWS	Vitamin E	< 0.2%
3	21	1		Vitamin C	< 0.2%
3	21	1	10% DIET	Control	< 0.2%
3	21	1	COWS	Vitamin E	< 0.2%
3	21	1		Vitamin C	< 0.2%
3	21	3	CONTROL	Control	< 0.2%
3	21	3	COWS	Vitamin E	< 0.2%
3	21	3		Vitamin C	< 0.2%
3	21	3	10% DIET	Control	< 0.2%
3	21	3	COWS	Vitamin E	< 0.2%
3	21	3		Vitamin C	< 0.2%
3	21	7	CONTROL	Control	< 0.2%
3	21	7	COWS	Vitamin E	< 0.2%
3	21	7		Vitamin C	< 0.2%
3	21	7	10% DIET	Control	< 0.2%
3	21	7	COWS	Vitamin E	< 0.2%
3	21	7		Vitamin C	< 0.2%
3	28	1	CONTROL	Control	< 0.2%
3	28	1	COWS	Vitamin E	< 0.2%
3	28	1		Vitamin C	< 0.2%
3	28	1	10% DIET	Control	< 0.2%
3	28	1	COWS	Vitamin E	< 0.2%
3	28	1		Vitamin C	< 0.2%
3	28	3	CONTROL	Control	< 0.2%
3	28	3	COWS	Vitamin E	< 0.2%
3	28	3		Vitamin C	< 0.2%
3	28	3	10% DIET	Control	< 0.2%
3	28	3	COWS	Vitamin E	< 0.2%
3	28	3		Vitamin C	< 0.2%
3	28	7	CONTROL	Control	< 0.2%
3	28	7	COWS	Vitamin E	< 0.2%
3	28	7		Vitamin C	< 0.2%
3	28	7	10% DIET	Control	< 0.2%
3	28	7	COWS	Vitamin E	< 0.2%
3	28	7		Vitamin C	< 0.2%

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REFERENCES

1. Alvarez, V. B. 2009. Chapter 5: Fluid Milk and Cream Products. Pages 73-133 in The Sensory Evaluation of Dairy Products. S. Clark, M. Costello, M. Drake, and F. Bodyfelt, ed. Springer Science + Business Media, LLC, New York, NY.

2. Anderson, J. L., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. J. Dairy. Sci. 89: 3133-3142.

3. Ardt, M. V., S. E. Duncan, J. E. Marcy, T. E. Long, S. F. O'Keefe, and S. R. Nielsen-Sims. 2005. Effect of antioxidant (α-tocopherol and ascorbic acid) fortification on light-induced flavor of milk. J. Dairy Sci. 88:872-880.

4. Barrefors, P., K. Granelli, L-Å. Appelqvist, and L. Bjoerck. 1995. Chemical characterization of raw milk samples with and without oxidative off-flavor. J. Dairy Sci. 78:2691-2699.

5. Bitman, J. and D.L. Wood. 1990. Changes in milk fat phospholipids during lactation. J. Dairy Sci. 73:1208-1216.

6. Bodyfelt, F. W., J. Tobias, and G. M. Trout. 1988. The Sensory Evaluation Of Dairy Products. Van Nostrand Reinhold, New York, NY.

7. Bruhn, J.C., A. A. Franke, and G. S. Goble. 1976. Factors relating to development of spontaneous oxidized flavor in raw milk. J. Dairy Sci. 59:828-833.

8. Cerbulis, J., and H. M. Farrell. 1975. Composition of milks of dairy cattle. I. Protein, lactose, and fat contents and distributions of protein fraction. J. Dairy Sci. 58(6):817-827.

9. Cervato, G., R. Cazzola, and B. Cestaro. 1999. Studies on the antioxidant activity of milk caseins. Int. J. Food Sci. Nutr. 50:291-296.

10. CFR (Code of Federal Regulations). 2012. Title 21, Sec. 131.110. Milk. Available: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.110. Accessed on Feb. 16, 2013.

11. Charmley, E. and J. W. G. Nicholson. 1993. Injectable α-tocopherol for control of oxidized flavour in milk from dairy cows. Can. J. Anim. Sci. 73:381-392.

12. Charmley, E., and J. W. G. Nicholson. 1994. Influence of dietary fat source on oxidative stability and fatty acid composition of milk from cows receiving a low or high level of dietary vitamin E. Can. J. Anim. Sci. 74:657-664.

المنارة للاستشارات

www.manaraa.com

13. Christie, W.W. 1995. Composition and Structure of Milk Lipids. Page 1-16 in Advanced Dairy Chemistry, Vol. 2. P. F. Fox, ed. Chapman & Hall, London, UK.

14. Clark, S., M. Costello, M. Drake, and F. Bodyfelt. 2009. The Sensory Evaluation of Dairy Products. 2nd. Springer Science + Business Media, LLC, New York, NY.

15. Corbett, W. J., and P. H. Tracy. 1943. The incidence of oxidized flavor in the ilk of individual cows within one herd. J. Dairy Sci. 25:1095-1106.

16. Coupland, J. N., and D. J. McClements. 1996. Lipid oxidation in food emulsions. Trends Food Sci. Technol. 7(3):83-91.

17. Dimakou, C., S. kiokias, I. Tsaprouni, and V. Oreopoulou. 2007. Effect of processing and storage parameters on the oxidative deterioration of oil-in-water emulsions. Food Biophysics. 2(1):38-45.

18. Dimick, P. S. 1976. Effect of fluorescent light on repartition of riboflavin in homogenized milk. J. Dairy Sci. 59:2019-2023.

19. Dunkley, W. L. and A. A. Franke. 1967. Evaluating susceptibility of milk to oxidized flavor. J. Dairy Sci. 50:1-9.

20. Dunkley, W. L., A. A. Franke, J. Robb, and M. Ronning. 1968. Influence of dietary copper and ethylenediaminetetraacetate on copper concentration and oxidative stability of milk. J. Dairy Sci. 51:863-866.

21. Ellis, K. A., G. Innocent, D. Grove-White, P. Cripps, W. G. McLean, C. V. Howard, M. Mihm. 2006. Comparing the fatty acid composition of organic and conventional milk. J. Dairy Sci. 89:1938-1950.

22. ERS. 2012. Dairy products: Per capita consumption, United States (Annual). Dairy Data. USDA-ERS. Available: http://www.ers.usda.gov/datafiles/Dairy_Data/pcconsp_1_.xls. Accessed on Mar. 7, 2013.

23. ERS. 2012. Fluid milk sales by product (Annual). Dairy Data. USDA-ERS. Available: http://www.ers.usda.gov/datafiles/Dairy_Data/fluidsales_1_.xls. Accessed on Mar. 7, 2013.

24. Fearon, A. M., C. S. Mayne, and C. T. Charlton. 1998. Effect of naked oats in the dairy cow's diet on the oxidative stability of milk fat. J. Sci. Food Agric. 76:546-553.

25. Fearon, A. M., C. S. Mayne, J. A. M. Beattie, and D. W. Bruce. 2004. Effect of level of oil inclusion in the diet of dairy cows at pasture on animal performance and milk composition and properties. J. Sci. Food Agric. 84:497-504.

المنارة للاستشارات
www.manaraa.com

26. Focant, M., E. Mignolet, M. Marique, F. Clabots, T. Breyne, D. Dalemans, and Y. Larondelle. 1998. The effect of vitamin D supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. J. Dairy Sci. 1:1095-1101.

27. Fox, P. F. 1983. Developments in dairy chemistry-2. Applied Science Publishers LTD, Essex, UK.

28. Fox, P. F., and P. L. H. McSweeney. 1998. Dairy chemistry and biochemistry. Blackie Academic & Professional, London, UK.

29. Frankel, E. N. 1998. Antioxidant. Page 129-166 in Lipid Oxidation. The Oily Press Ltd, Dundee, Scotland.

30. Grandelli, K., P. Barrefors, L. Björck, and L-Å. Appelqvist. 1998. Further studies on lipid composition of bovine milk in relation to spontaneous oxidized flavour. J. Sci. Food Agric. 77:161-171.

31. Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244-3257.

32. Haase, G. and W. L. Dunkley. 1969. Ascorbic acid and copper in linoleate oxidation. II. Ascorbic acid and copper as oxidation catalysts. J Lipid Res. 10:561-567.

33. Harding, F. 1995. Milk quality. Blackie Academic & Professional, London, UK.

34. Havemose, M. S., M. R. Weisbjerg, W. L. P. Bredie, and J. H. Nielsen. 2004. Int. Dairy J. 14:563-570.

35. Havemose, M. S., M. R. Weisbjerg, W. L. P. Bredie, H. D. Poulsen, and J. H. Nielsen. 2006. J. Dairy Sci. 89:1970-1980.

36. Hedegaard, R. V., D. Kristensen, J. H. Nielson, M. B. Frøst, H. Østdal, J. E. Hermansen, M. Kröger-Ohlsen, and L. H. SKibsted. 2006. Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk. J. Dairy Sci. 89:495-504.

37. Herrington, B. L., J. W. Sherbon, R. A. Ledford, and G. E. Houghton. 1972. Composition of milk in New York State. New York's Food Life Sci. Bull. 18:1-23.

38. Hoffman, L., and A. Baker. 2010. Market Issues and Prospects for U.S. Distillers' Grains Supply, Use, and Price Relationships. FDS: Feed Outlook. FDS-10k-01. ERS-USDA. Available: http://www.ers.usda.gov/media/107533/fds10k01_1_.pdf. Accessed Mar. 7, 2013.

المنارة للاستشارات

www.manaraa.com

39. Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91:3544-3553.

40. Jenness, R. and R. E. Sloan. 1970. The composition of milks of various species. A review. Diary Sci. Abstr. 32:599-612.

41. Jensen, R. G., A. M. Ferris, and C. J. Lammi-Keefe. 1991. The composition of milk fat. J. Dairy Sci. 74:3228-3243.

42. Jensen, R. G. 1995. Handbook of milk composition. Academic Press, Inc., San Diego, CA.

43. Jensen, R. G. 2002. The composition of bovine milk lipid: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

44. Juhlin, J., F. Fikse, A. Lunden, J. Pickova, and S. Agenas. 2010. Relative impact of α-tocopherol, copper and fatty acid composition on the occurrence of oxidized milk flavor. J. Diary Res. 77:302-309.

45. Kennelly, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60:137-152.

46. Kim, H. J. 2007. Oxidation mechanism of riboflavin destruction and antioxidant mechanism of tocotrienols. Ph.D. Diss., Ohio State Univ., Columbus, OH.

47. Kim, T. S., E. A. Decker, and J. H. Lee. 2012. Antioxidant capacities of α-tocopherol, trolox, ascorbic acid, and ascorbyl palmitate in riboflavin photosensitized oil-in-water emulsions. J. Food Chem. 133:68-75.

48. Kim, Y. D., and C. V. Morr. 1996. Dynamic headspace analysis of light activated flavor in milk. Int. Dairy J. 6:185-193.

49. Klei, L. R., J. M. Lynch, D. M. Barbano, P. A. Oltenacu, A. J. Lednor, and D. K. Bandler. 1997. Influence of milking three times a day on milk quality. J. Dairy Sci. 80:427-436.

50. Kononoff, P. J. 2006. Does Feeding Distillers Grains to Dairy Cows Affect Milk Components? NebGuide G1679. Univ. of Nebraska-Lincoln Extension. Available: http://ianrpubs.unl.edu/epublic/live/g1679/build/g1679.pdf. Accessed Mar. 7, 2013.

51. Leonardi, C., S. Bertice, and L. E. Armentano. 2005. Effect of Increasing Oil from Distillers Grains or Corn Oil on Lactation Performance. J. Dairy. Sci. 88: 2820-2827.

المنارة للاستشارات

www.manaraa.com

52. Let, M. B., C. Jacobsen, and A. S. Meyer. 2005. Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. Int. Dairy J. 15:173-182.

53. Lindmark-Månsson, H. 2008. Fatty acids in bovine milk fat. Food Nutr Res. Vol. 52. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2596709/pdf/FNR-52-1821.pdf. Accessed on Mar. 14, 2013.

54. Lindmark-Månsson, H., and B. Åkesson. 2000. Antioxidative factors in milk. Br. J. Nutr. 84:S103-S110.

55. Liu, K. S. 2011. Chemical Composition of Distillers Grains, a Review. J. Agric. Food Chem. 59: 1508-1526.

56. Liu, Q., J. Wang, D. Bu, Khas-Erdene, K. Liu, H. Wei, L. Zhou, and D. C. Beitz. 2010. Influence of linoleic acid content on the oxidation of milk fat. J. Agric. Food Chem. 58:3741-3746.

57. Loosli, J. K., K. L. Turk, and F. B. Morrison. 1952. The value of distillers feeds for milk production. J. Dairy Sci. 35(10): 868-873.

58. Manitoba Agriculture, Food and Rural Initiatives. Troubleshooting oxidized flavour problems in bulk milk. Manitoba Agriculture, Food and Rural Initiatives. Government of Manitoba. Available: http://www.gov.mb.ca/agriculture/livestock/dairy/cda20s02.html. Accessed on Feb. 16, 2013.

59. Mathews, H. K., and M. J. McConnell. 2009. Ethanol co-product use in U.S. cattle feeding: lessons learned and considerations. FDS-09D-01. ERS-USDA. Available: http://www.ers.usda.gov/media/147398/fds09d01.pdf. Accessed on Mar. 14, 2013.

60. Morales, M. S., D. L. Palmquist., and W. P. Weiss. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. J. Dairy Sci. 83:2112-2119.

61. Mortensen, G., G. Bertelsen, B. K. Mortensen, and H. Stapelfeldt. 2004. Light-induced changes in packaged cheese – a review. Int. Dairy J. 14:85-102.

62. Nichols, J. R., D. J. Schingoethe, H. A. Maiga, M. J. Brouk, and M. S. Piepenbrink. 1998. Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. J. Dairy Sci. 81:482-491.

63. Nicholson, J. W. G., and A. M. St-Laurent. 1991. Effect of forage type and supplemental dietary vitamin E on milk oxidative stability. Can. J. Anim. Sci. 71:1181-1186.

المنارة للاستشارات

www.manaraa.com

64. O'Connor, T. P., and N. M. O'Brien. 2006. Lipid Oxidation. Pages 557-600 in Advanced Dairy Chemistry. Vol. 2. P. F. Fox, and P. L. H. McSweeney, ed. Springer Science + Business Media, LLC, New York, NY.

65. Offer N. W., M. Marsden, J. Dixon, B. K. Speake, and F. E. Thacker. 1999. Effect of dietary fat supplements on levels of n-3 polyunsaturated fatty acids, trans acids and conjugated linoleic acid in bovine milk. Anim Sci. 69:615-625.

66. Østdal, H., M. J. Bjerrum, J. A. Pedersen, and H. J. Andersen. 2000. Lactoperoxidase-induced protein oxidation in milk. J. Agric. Food Chem. 48:3939-3944.

67. Palmquist, D. L. and T. C. Jenkins. 1980. Fat in lactation rations: Review. J. Dairy Sci 63:1-14.

68. Palmquist, D. L. A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771.

69. Pantoja, J., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 1994. Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows. J. Dairy Sci. 77:2341-2356.

70. Powers, W. J., H. H. Van Horn, and B. Harris, JR. 1995. Effects of variable sources of distillers dried grains plus solubes on milk yield and composition. J. Dairy Sci. 78:388-396.

71. Rausch, K. D., and R. K. Belyea. 2006. The future of coproducts from corn processing. Applied Biochem. and Biotechnol. 128:47-85.

72. Sasikala-Appukuttan, A. K., D. J. Schingoethe, A. R. Hippen, K. F. Kalscheur, K. Karges, and M. L. Gibson. 2008. The feeding value of corn distillers solubles for lactating dairy cows. J. Dairy Sci. 91:279-287.

73. Schaich, K. M. 1980. Free radical initiation in proteins and amino acids by ionizing and ultraviolet radiations and lipid oxidation. Part III. Free radical transfer from oxidizing lipids. CRC Crit. Rev. Food Sci. Nutr. 13:189-244.

74. Schingoethe, D. J., M. J. Brouk, and C. P. Birkelo. 1999. Milk Production and Composition from Cows Fed Wet Corn Distillers Grains. J. Dairy. Sci. 82:574-580.

75. Schingoethe, D. J. 2001. Feeding Wet Distillers Grains to Dairy Cattle. Proc. Distillers Grains Technol. Council. 5th Annu. Sym., Louisville, Ky. May 23-24.

76. Schingoethe, D. J. 2006. Utilization of DDGS by Cattle. Pages 61-74 in Proc. 27th Western Nutrition Conf., Winnipeg, Manitoba, Canada. September 19-20.

المنارة للاستشارات
www.manaraa.com

77. Schingoethe, D. J., K. F. Kalscheur, A. R. Hippen, and A. D. Garcis. 2009. Invited review: the use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802-5813.

78. Shipe. W. F., R. Bassette, D. D. Deane, W. L. Dunkley, E. G. Hammond, W. J. Harper, D. H. Kleyn, M. E. Morgan, J. H. Nelson, and R. A. Scanlan. 1978. Off-flavors of milk: Nomenclature, standards and bibliography. J. Dairy Sci. 61:855-869.

79. Singleton, J. A., L. W. Aurand, and F. W. Lancaster. 1963. Sunlight flavor in milk. I. A study of components involved in the flavor development. J. Dairy Sci. 46:1050-1053.

80. Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80: 2639-2645.

81. Stapelfeldt, H., K. N. Nielsen., S. K. Jensen., and L. H. Skibsted. 1999. Free radial formation in freeze-dried raw milk in relation to its α-tocopherol level. J. Dairy Sci. 66:461-466.

82. Sugiarto M., A. Ye, M. W. Taylor, and H. Singh. 2010. Milk protein-iron complexes: inhibition of lipid oxidation in an emulsion. Dairy Sci. Technol. 90:87-98.

83. Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801-2814.

84. Taylor, M. J. and T. Richardson. 1980. Antioxidant activity of skim milk: effect of heat and resultant sulfhydryl groups. J. Dairy Sci. 63: 1783-1795.

85. Timmons, J. S., W. P. Weiss, D. L. Palmquist, and W. J. Harper. 2001. Relationships among dietary roasted soybeans, milk components, and spontaneous oxidized flavor milk. J. Dairy Sci. 84:2440-2449.

86. Tong, L. M., S. Sasaki, D. J. McClements, and E. A. Decker. 2000. Mechanism of antioxidant activity of a high molecular weight fraction of whey. J. Agric. Food Chem. 48:1473-1478.

87. USDA-ERS. 2012. Dairy Background. Available: http://www.ers.usda.gov/topics/animal-products/dairy/background.aspx. Accessed on Mar. 7, 2013.

88. USDA-NASS. 2013. Milk: Production by Year, US. Available: http://www.nass.usda.gov/Charts_and_Maps/Milk_Production_and_Milk_Cows/milkprod.asp. Accessed on Mar. 7, 2013.

89. Walstra, P., and R. Jenness. 1984. Dairy chemistry and physics. John Wiley & Sons, Inc., New York, NY.

المنارة للاستشارات

www.manaraa.com

90. Waraho, T., D. J. McClements, and E. A. Decker. 2011. Mechanisms of lipid oxidation in food dispersions. Trends Food Sci. Technol. 22:3-13.

91. Wong, N. P., R. Jenness, M. Keeney, and E. H. Marth. 1988. Fundamentals of dairy chemistry. 3rd. Von Nostrand Reinhold Company, New York, NY.

92. Yoshida, Y., and E. Niki. 1992. Oxidation of methyl linoleate in aqueous dispersions induced by copper and iron. Arch. Biochem. Biophys. 295(1):107-114.

المنارة للاستشارات
www.manaraa.com