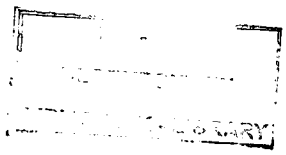

Evaluation of bacterial fermentation and synthetic fortification as a means to enrich yogurt with conjugated linoleic acid

by

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Submitted in fulfilment of the requirements
for the degree of

MAGISTER SCIENTIAE
(FOOD SCIENCE)

In the

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Faculty of Natural and Agricultural Sciences
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January 2012

DECLARATION

I declare that the dissertation hereby submitted by me for the MSc. Food Science degree in the Faculty of Natural and Agricultural Science at the University of the Free State is my own independent work and has not previously been submitted by me at another university/ faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

T. J. Burger

January 2012

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ACKNOWLEDGEMENTS

My sincere gratitude and appreciation goes to the following persons and institutions for their contributions to the completion of this study:

God Almighty who gave me the knowledge, hope and strength during this study;

Dr. J. Myburgh, for his input, encouragement and time devoted during this study;

Prof. A. Hugo, for his inputs, devoted time and constructive criticism of the manuscript;

Prof. C. J. Hugo, for her encouragement, support and assistance;

Dairybelle, Bloemfontein for yogurt supply and packaging;

Mr. E. Slabber for his time and help with sample preparations;

SAMPRO for the bursary;

Fellow post-graduate students for all their support and encouragement;

Eileen Roodt, Lize Liebenberg and Cobus Ferreira for their help and assistance with the analysis;

My parents and other loved ones for constant interest, encouragement and support.

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LIST OF ABREVIATIONS

ANOVA	Analysis of variance
A_w	Water activity
cfu	Colony forming units
CLA	Conjugated linoleic acid
CLA1	C18:2c9t11
CLA2	C18:2t10c12
CPS	Centipoise
C16:1	C16:1c9
C17:1	C17:1c10
C18:2	C18:2c9,12
C20:3	C20:3c11,14,17
C20:4	C20:4c5,8,11,14
C24:1	C24:1c15
°C	Degrees Celcius
EC	Estimated counts
EFC	Extractable fat content
e.g.	For example
et al	(*et alii*) and others
FA	Fatty acid
FAME	Fatty acid methyl esters
FFA	Free fatty acid

FFDM	Fat free dry matter
Fig	Figure
g	gram
GC	Gas chromatograph
HDL	High density lipoprotein
HTST	High temperature short time
kg	Kilogram
LA	Linoleic acid
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
m	Meter
MFG	Milk fat globules
mg	Milligram
min	Minute
ml	Millilitre
mPa	Mega Pascal
MRS	de Man-Rogosa-Sharpe
MUFA	monounsaturated fatty acid
µl	Microliter
µm	Micrometer
N	Normal
NaOH	Sodium hydroxide
NS	Not significant
PUFA	Polyunsaturated fatty acid

RDA	Recommended dietary allowance
rpm	Revolutions per minute
sec	Second
SFA	Saturated fatty acid
SFO	Sunflower oil
SPC	Standard plate count
ssp	Sub-species
TBARS	Thiobarbituric acid reactive substances
TG	Triglyceride
TEFC	Total extractable fat content
TNTC	Too numerous to count
Tonalin®	Tonalin® 60-WDP
TS	Total solids
UFA	Unsaturated fatty acid

CHAPTER 1

INTRODUCTION

The demand for foods with high value and added health benefits continuously increases (Ip *et al.*, 1991). Consumers have an increasing desire to take a more proactive role in optimizing personal health and well-being, without relying on pharmaceuticals (Champagne & Mollgaard, 2008).

Functional foods are foods or food components that are scientifically recognized as foods with physiological benefits beyond those of basic nutrition (Gibson & Williams, 2000). Growing consumer interest in the role of nutrition for health and well-being is the primary driver behind the success of the functional food market (Gagada *et al.*, 1999).

The dairy-based beverages market may be seen as the market of main focus when the sales of yogurt, milk and other beverages are taken into consideration. Dairy beverages containing probiotics and / or prebiotics dominate the functional dairy beverage market (Gagada *et al.*, 1999). The focus in the dairy-based functional food marker has recently moved to conjugated linoleic acid (CLA) fortified dairy products (Gagada *et al.*, 1999). There is a substantial need for CLA enriched dairy products (Parodi, 1999).

The health benefits of CLA may relate to specific isomers only (Peterson *et al.,* 2002). In studies by Ha *et al.* (1990) and Ip *et al.* (1991) the cancer inhibiting properties of CLA in mice and rats were studied. They found that all the CLA were incorporated into tissue triacylglycerols, but the *cis*-9, *trans*-11 (CLA1) and *trans*-10, *cis*-12 (CLA2) isomers were incorporated into the membrane phospholipids and are therefore assumed to be the most biologically active CLA isomers (Ha *et al.*, 1990; Ip *et al.*, 1991). The *cis*-9, *trans*-11 isomer (CLA1) is known for its anticancer properties and CLA2 is effective in controlling body weight (Brown & McIntosh, 2003; Park & Parize, 2007). CLA at near-physiological concentrations inhibits tumor genesis, independently of the amount and type of fat in the diet (Parodi, 1999).

Conjugated linoleic acid isomers have also been found in bovine milk (Jensen *et al.*, 1991). Although dairy is the richest natural source of CLA, the level of CLA is still very low in dairy products. The average level of CLA present in dairy is in the range of 0.55 – 9.12 mg/g fat, depending on the specific product (Akalin *et al.*, 2007).

The current estimated daily intake of CLA from food sources is in the range of 150 mg to 1.5 g per day. These values are dependent on gender and vary among individuals (Rodríguez-Alcalá & Fontecha, 2007). The recommended dietary allowance (RDA) of CLA is much more than what is present in dairy products. In recent years a number of strategies have been initiated to produce dairy products with elevated CLA levels (Hur *et al.*, 2007).

It has been found that certain starter cultures used in dairy processing have the ability to produce CLA since it was discovered that fermented dairy products contain higher levels of CLA than non-fermented dairy products (Prandini *et al.,* 2007). Further studies proved that it is linoleic acid (LA) isomerase-containing microorganisms that are capable of isomerizing LA to CLA during fermentation (Lin e*t al.*, 1999; Prandini *et al.,* 2007). Starter cultures that were identified with this capability included *Propionibacterium, Lactobacillus, Lactococcus, Streptococcus* and *Bifidobacterium* (Prandini *et al.,* 2007).

Linoleic acid can be added to media to be converted to CLA by certain strains of microorganisms used as starter cultures (Sieber *et al.*, 2004). Sunflower oil (SFO) is one example of oil that can be used as a cheaper alternative source of LA in media (Sieber *et al.*, 2004).

Dairy products can also be synthetically fortified with CLA. CLA is also available in an encapsulated powder form. This form of synthetic CLA mixes easier with dairy products than the CLA in oil form (Jimenez *et al.*, 2008). Care should be taken in the amount of powder added to the yogurt. A too high amount can lead to a powdery or cardboardy taste, excessive firmness and a grainy texture (Mistry & Hassan, 1992; Guzmán-González *et al.*, 2000).

In the first phase of this study the ability of frequently used yogurt starter culture mixtures to convert LA to CLA were evaluated. After fermentation with SFO or LA at levels

equivalent to a LA content of 1 mg/ml yogurt, CLA levels were monitored in the yogurt. The CLA levels obtained in these treatments were compared to the RDA for CLA.

In the second phase of this study direct fortification of yogurt with synthetic CLA was evaluated in an attempt to create a product that would deliver an amount of CLA in one portion of yogurt closer to the RDA for CLA. The sensory, chemical and physical properties of the CLA enriched yogurt were investigated to evaluate the impact of the fortification methods on the natural yogurt composition and quality.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Milk is a complex colloidal dispersion of fat globules and proteins (casein and whey) in an aqueous solution of lactose, minerals and other minor constituents. Milk is composed of water and milk solids (Table 2.1). Milk fat is a natural fat and is unique in its physical, chemical and biological properties (Singh *et al.,* 1997).

Table 2.1: ***General composition of bovine milk*** **(Jensen *et al.*, 1991)**

Protein	3.2 %
Casein*	2.6 %
Fat	3.9 %
Lactose	4.6 %
Total solids	12.7 %
Ash	0.7 %
Water	87 %
Energy (kJ/100ml)	277

**Thus approximately 80% of the protein.*

Conjugated linoleic acid (CLA) isomers with anti-carcinogenic activity have been found in bovine milk (Fogerty *et al.*, 1988; Ha *et al.*, 1989). The CLA isomer that is being referred to in this literature study is the C18:2c9t11 (CLA1) isomer, unless stated otherwise. This specific isomer of CLA is known to be the most biologically active form of CLA. The reason for this is the fact that CLA is not incorporated into the phospholipid fraction of the tissue after the consumption of CLA containing foods (Jiang *et al.*, 1998). The CLA content in milk fat can be increased by increasing the polyunsaturated fatty acids (PUFA's) in the cow's diet (German & Dillard 1998).

Conjugated linoleic acid was discovered accidently by Pariza and Hargraves in the 80's while they were investigating the carcinogenic properties of grilled beef. Contrary to their expectations, the fatty acids (FA's) present in grilled beef exhibited anti-carcinogenic, rather than pro-carcinogenic properties. Ever since that discovery several tests using animal models and cell cultures derived from human and animal cells, showed beneficial effects in health-related disorders and CLA isomers have been shown to have anti-adipogenic, anti-carcinogenic, anti-atherogenic, anti-diabetogenic and anti-inflammatory properties (Bhattacharye *et al.*, 2006).

The CLA in milk fat is only a portion of the total CLA after biohydrogenation, as CLA takes part as an intermediate in rumen biohydrogenation by the rumen bacteria in the formation of vaccenic acid and stearic acid. The amount of CLA present in the milk fat is dependent on the rumen bacteria's production of vaccenic acid (*trans*-11 $C_{18:1}$) and CLA, as well as the activity of Δ^9-desaturase enzyme (Peterson *et al.*, 2002).

Although dairy is the richest natural source of CLA, the level of CLA is still very low in dairy products (Akalin *et al.*, 2007). A number of factors such as the processing conditions can influence the CLA concentration in the final milk or dairy product (Campbell *et al.*, 2003), but it depends mainly on the initial CLA level of the raw milk (Bauman *et al.*, 2000; Collomb *et al.*, 2006). The CLA concentration in milk can be increased by modifying the cow's diet. To reach the recommended dietary level of CLA, 30 servings (1 serving = 200 ml) of this naturally increased CLA milk must be consumed; therefore options of direct fortification of dairy must rather be considered (Campbell *et al.*, 2003).

Before any research on direct CLA fortification can be done, the consumers' perception towards fortified products and their willingness to pay for such products must be taken into consideration. Research confirmed that consumers are willing to pay for CLA enriched products (Jimenez *et al.*, 2008), especially individuals with a history of cancer (Campbell *et al.*, 2003).

Growing consumer interest in the role of nutrition for health and well-being is the primary driver behind the success of the functional food market. Functional foods are foods or food components that are scientifically recognized as foods with physiological benefits beyond those of basic nutrition (Gibson & Williams, 2000). In this study the focus will be on CLA fortified yogurt.

Consumers have an increasing desire to take a more pro-active role in optimizing their personal health and well-being, without relying on pharmaceuticals (Champagne & Mollgaard, 2008). The rising medical costs of the past few years forced people to find cheaper and more effective means of protecting their health, and therefore the interest in functional food products has increased (Richardson, 1996).

The dairy-based beverages market may be seen as the market of main focus when the sales of yogurt, milk and other dairy beverages are taken into consideration. Dairy beverages containing probiotics and or prebiotics dominate the functional dairy beverage market. Recently the focus also moved to CLA fortified dairy products (Gagada *et al.*, 1999).

2.2 BASIC COMPOSITION OF MILK FATS

The general composition of bovine milk is listed in Table 2.1. Although the milk composition is altered through different factors such as the breed of cow and stage of lactation etc., the commercial market product is consistently uniform because of pooling, standardization of fat content and exclusion of colostral and mastitis milk (Jensen *et al.*, 1991).

Bovine milk contains approximately 3.5 % to 5 % of total lipid, existing as emulsified globules coated with a membrane derived from the secretory cell membrane (Jensen *et al.*, 1991; Belitz & Grosch, 2008). Lipids are bio-organic molecules that are hydrophobic. In other words, they do not mix with or dissolve in water. Among lipids there is a category known as "fats". Lipids are referred to as “fat” when solid at room temperature as opposed to “oil” which is liquid at room temperature (25°C). Each fat molecule is comprised of a glycerol (alcohol) molecule and at least one FA (hydrocarbon chain with an acid group attached) (Belitz & Grosch, 2008).

The long chain FAs present in the milk originate from the enzyme activity of the rumen bacteria and are then transported to the secretory cells via the blood and lymph or from synthesis in the secretory cells (Belitz & Grosch, 2008). Milk FA composition has a number of effects on the milk quality, including aspects such as its physical properties as well as its

nutritional properties. The FA composition of the milk also affects the organoleptic properties of milk (Chilliard *et al.*, 2000).

Bovine milk fat is unique in its composition, due to the great diversity of FA's (Palmquist, 2007). Approximately 400 different types of FA's are found in milk fat (MacGibbon & Taylor, 2006). The diversity arises from the effects of ruminal biodegradation on dietary FAs and the range of FA's synthesized *de novo* in the mammary gland (Palmquist, 2007). *De novo* synthesis results in short chain FA's and medium chain FA's and accounts for approximately 45 % (w/w) of the total FA's in milk fat (MacGibbon & Taylor, 2006).

The major long chain FA's occurring in milk are: myristic-, palmitic-, stearic and oleic acids and the major short chain FA's are: butyric-, caproic-, caprylic- and capric acid (Palmquist, 2007). Generally full cream milk consists of approximately 3.4 % total fat, of which 2.5 % saturated fatty acids (SFA's), 0.85 % monounsaturated fatty acids (MUFA's), 0.1 % polyunsaturated fatty acids (PUFA's), 0.08 % omega-6 FA's and 0.02 % are omega-3 FA's (Belitz & Grosch, 2008). Approximately 95 % of the UFA's in milk fat is in the form of oleic acid, linoleic acid (LA) and α-linolenic acid, the precursors of CLA1 (Mallia *et al.*, 2008). Low fat milk consists of 2.0 % total fat, of which 1.3 % SFA's; 0.59 % MUFA's; 0.07 % PUFA's; 0.06 % omega-6 FA's and 0.01 % omega-3 FA's (Belitz & Grosch, 2008). Small amounts of mono-, diglycerides and free fatty acids (FFA's) may be present as a product of early lipolysis or incomplete synthesis (MacGibbon & Taylor, 2006).

Approximately 98 % of milk lipids are triacylglycerides (TG's) (Jensen *et al.*, 1991). Triacylglycerides are comprised of a glycerol backbone binding up to three different FA's which are composed of a hydrocarbon chain and a carboxyl group (Fig. 2.1) (Belitz & Grosch, 2008).

The distribution of FA's on the three *sn* positions of the triglyceride is not random (Jensen *et al.*, 1991). Bovine milk TG has a unique structure, much of the C4:0 to C10:0 are at the sn-3 position. Most of the C12:0 to C14:0 FA's are at the *sn*-2 position and most of the long chain FA's (C16:0 to C18:0) are bound at the sn-1 position (Jensen *et al.*, 1991). There are hundreds of different combinations of the distribution of the FA's on the TG chain. The pattern of the FA's is important when determining the physical properties of the lipids (Belitz & Grosch, 2008).

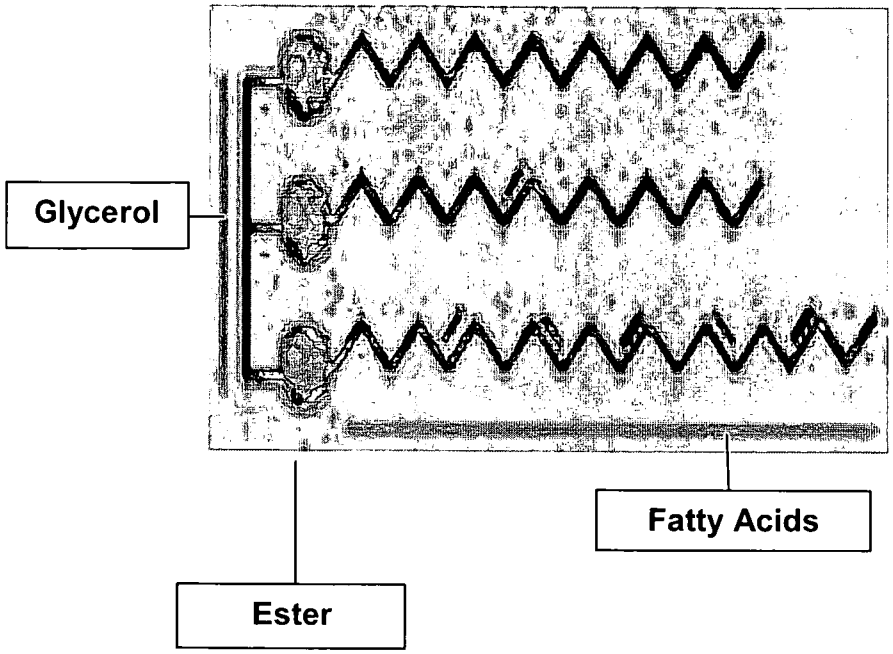


Figure 2.1 The triglyceride (TG) chemical structure (Belitz & Grosch, 2008).

Bovine milk contains 0.8 % of phospholipids. Phospholipids are mainly found in the milk FGM and they play a major role in structure due to their amphiphilic properties. Phospholipids are the main source of long-chain PUFA's (MacGibbon & Taylor, 2006). Cholesterol comprises 0.3 % of the milk fat (MacGibbon & Taylor, 2006).

2.3 CONJUGATED LINOLEIC ACID

CLA is a group of polyunsaturated fatty acids (PUFA's), existing as a mixture of positional and geometric isomers of octadecadienoic acid [linoleic acid (LA), 18:2n-6] (Chin *et al.*, 1992; Lin *et al.*, 1995; Parodi, 1997). Conjugated linoleic acid has the same chain length as its precursor, LA. The only difference is that the double bonds in CLA are conjugated, which is not the case in LA. Only one single carbon bond separates the conjugated bonds in CLA (Hur *et al.*, 2007). Conjugated linoleic acid differs from LA in that there is no methylene group separating the double bonds as in LA (Bhattacharye *et al.*, 2006).
Conjugated linoleic acid isomers have been found in bovine milk (Jensen *et al.*, 1991). Although 18 different isomers of CLA do exist (Peterson *et al.*, 2002), the C18:2c9t11 and the C18:2t10c12 (CLA2) isomers are the most common forms of CLA found in nature (Fritsche *et al.*, 1999; Brown & McIntosh, 2003; Hur *et al.*, 2007; Park & Parize, 2007). These isomers are the two most biologically active forms of CLA (Peterson *et al,* 2002).

HEALTH IMPLICATIONS OF CONJUGATED LINOLEIC ACID CONSUMPTION

Our diets contribute to about one third of all cancer deaths (Bhattacharye *et al.*, 2006). Studies have shown that CLA has inhibited growth in a number of human cancer cell lines. Conjugated linoleic acid is anti-atherogenic, immune-modulating and growth-promoting (Bhattacharye *et al.*, 2006).

In studies by (Ha *et al.*, 1990; Ip *et al.*, 1991) they studied the cancer inhibiting properties of CLA in mice and rats. It was found that all the CLA were incorporated into the tissue TG's, but that the CLA1 and CLA2 isomers were not incorporated into the membrane phospholipids and are therefore assumed to be the most biologically active CLA isomers (Ha *et al.*, 1990; Ip *et al.*, 1991).

The health effects of CLA may relate to only specific isomers (Peterson *et al.,* 2002). The CLA1 isomer is known for its anti-cancer properties and the CLA2 isomer is effective in controlling body weight (Brown & McIntosh, 2003; Park & Parize, 2007). The mechanism, by which CLA inhibits certain cancer cell lines, is still uncertain, but many scientists suspect the following: CLA may act as an anti-oxidant, contributing to inhibition of nucleotide and protein synthesis, reduction of cell proliferative activity and inhibition of both DNA-adduct formation and carcinogen activation (Bhattacharye *et al.*, 2006). Conjugated linoleic acid at near-physiological concentrations inhibits tumor-genesis, independently of the amount and type of fat in the diet (Parodi, 1997).

Conjugated linoleic acid can decrease the body fat mass without significantly affecting body weight (Bhattacharye *et al.*, 2006) and increase the lean muscle mass as well (Akalin *et al.*, 2007). Conjugated linoleic acid decreased some atherogenic risk factors in healthy overweight women in a clinical study (Bhattacharye *et al.*, 2006). Not only does CLA depress total cholesterol, but it also lowers the low density lipoprotein (LDL) (negatively associated with human health): high density lipoprotein (HDL) (positively associated with human health) cholesterol levels (Lin *et al.*, 1999).

Tumor incidence and the number of tumors amongst rats that were given CLA-enriched butter were reduced by over 50 % (Bauman *et al.*, 2000). One study suggested that high-fat dairy foods and CLA reduced colorectal cancer by 13 % and the risk of distal colon cancer by 34 %.

Conjugated linoleic acid also has a positive effect on the human immune system and these are the reasons why dairy products became a strong recommendation as part of the human diet internationally (Bhattacharye *et al.*, 2006). The role of CLA in vitamin-A metabolism has also been reported (Carta *et al.*, 2002).

FORMATION OF CONJUGATED LINOLEIC ACID

There are two mechanisms by which CLA in milk and dairy products are formed: isomerization of LA and linolenic acids through a biohydrogenation pathway in the rumen and free radical isomerization of LA and linolenic acids during processing (Akalin *et al.*, 2007). Fig. 2.2 illustrates that the majority of CLA in milk is formed through endogenous synthesis by Δ^9- desaturase with the precursor being vaccenic acid (*trans*-11 $C_{18:1}$), which is formed in rumen biohydrogenation of PUFA's, such as LA (C18:2c9,12) and α-linolenic acid (C18:3c9,12,15) (Collomb *et al.*, 2006; Peterson *et al.*, 2002).

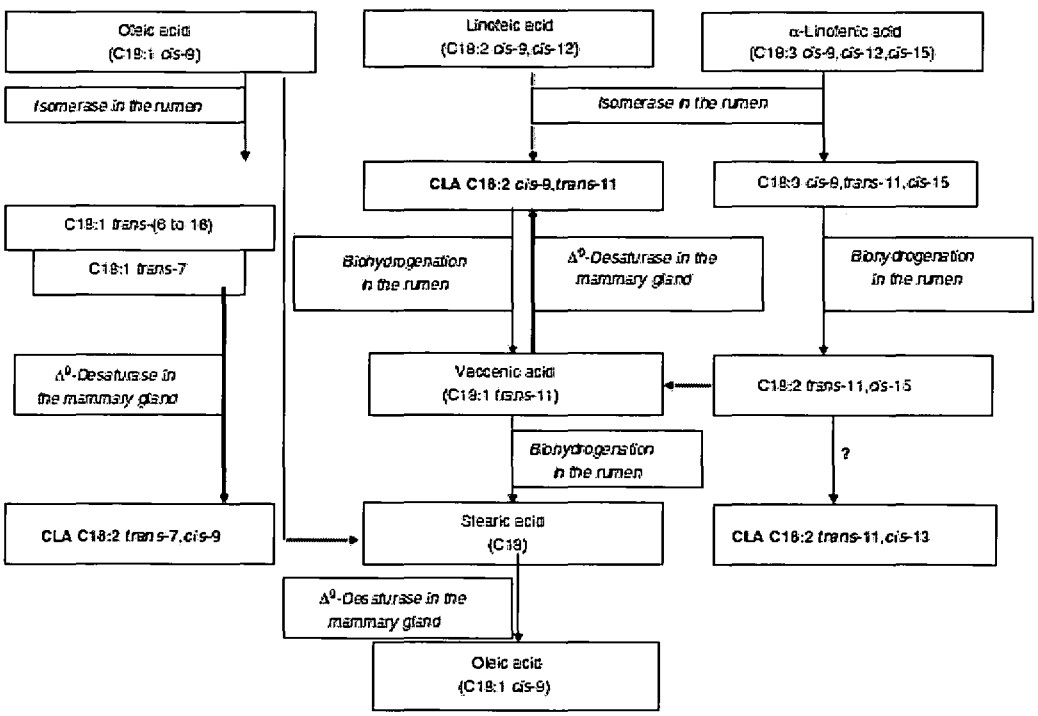


Figure 2.2 ***Known metabolic pathways for the formation of CLA isomers*** (Collomb *et al.*, 2006).

The CLA in milk fat is only a portion of the total CLA after biohydrogenation, as CLA isomers take part as intermediates in rumen biohydrogenation. The amount of CLA

present in the total fat of the milk is dependent on the rumen production of *trans*-11 $C_{18:1}$ and CLA, as well as the activity of Δ^9-desaturase (Peterson *et al*, 2002). Synthetic CLA is synthesized from LA by alkaline isomerization. Some isomers are also formed by low-temperature precipitation (Kim *et al.*, 2000; Kim, 2003).

CONJUGATED LINOLEIC ACID CONCENTRATION IN DAIRY PRODUCTS

Although dairy are the richest natural sources of CLA, the level of CLA is still very low in these products when the recommended dietary allowance (RDA) for CLA is considered. The average level of CLA present in dairy products is in the range of 0.55 to 9.12 mg/g fat, depending on the specific product (Akalin *et al.*, 2007).

Some fermented dairy products contain higher levels of CLA than non-fermented milk (Prandini *et al.*, 2007). Conjugated linoleic acid levels in cheeses are in the range of 3.59 to 7.96 mg/g of fat. Swiss, Blue, Brie and Edam cheeses have higher CLA levels than other cheese. More matured Cheddar also has a higher level than medium Cheddar, but not in statistically significant amounts (Lin *et al.*, 1999). The level of CLA in other fermented dairy products range from 3.82 to 4.66 mg/g of fat. The highest level is observed in cultured buttermilk (Lin *et al.*, 1999).

Non-fat frozen dairy dessert and non-fat yogurt was found to have a very low CLA content with values of approximately 0.6 mg and 1.7 mg/g of lipid respectively due to the low total fat content of non-fat dairy products (Lin *et al.*, 1999).

The values for CLA levels of selected dairy products are listed in Table 2.2. Variation among samples of the same product may occur. Processing, breed and diet are just some of the factors that may influence the CLA content, therefore the CLA in the food primarily depends on the CLA content of the raw product (Bell & Kennelly, 2001).

FACTORS INFLUENCING DAIRY CONJUGATED LINOLEIC ACID CONTENT

A number of factors can influence the CLA content of milk and other dairy products. These factors can be divided into three categories: diet related, animal related and post-harvest related (Campbell *et al.*, 2003; Khanal & Olson, 2004).

Table 2.2: ***CLA content of various Dairy products*** (Bell & Kennelly, 2001).

Foodstuff	Total CLA content (mg/g fat)
Homogenized milk	5.5
Butter fat	4.7
Mozzarella cheese	4.9
Plain yogurt	4.8
Ice cream	3.6

Animal and diet related:

Bovine diet

Seasonal fluctuations and specific breed of cow are animal-related factors that can influence the concentration of CLA in milk fat (Prandini *et al.,* 2007). Although diet can influence the CLA level in the milk significantly, the levels may still vary among individual cows fed the same diet. The CLA level variation amongst individuals may be attributed to rumen biohydrogenation and enzyme (desaturase) activity in the mammary gland (Peterson *et al.*, 2002).

The amount of PUFA's present is also an important component in the diet which have an effect on the final concentration of CLA in the milk (Peterson *et al.*, 2002). Soybean, which contains high levels of PUFA's included in the diet caused cows to produce milk with a slightly higher level of CLA. The soybean acts as a slow-release source of LA, which is then converted to CLA. Other oils which can act as potential LA sources are sunflower oil (SFO) or flaxseed oil which may also be incorporated in the diet (Peterson *et al.*, 2002).

Post-harvest related factors:

Microorganisms used in the manufacturing of fermented dairy products

The concentration of CLA in fermented dairy products may be influenced by the bacterial strain, number of bacterial cells, substrate concentration as well as the incubation period and pH (Prandini *et al.,* 2007). Many studies have proved that the choice of starter culture

played an important role in the final CLA level of the dairy product (O'Shea *et al.*, 2000). It was shown that certain starter cultures used in dairy processing, also had the ability to produce CLA. Conjugated linoleic acid is produced by starter cultures during lactic acid fermentation (Prandini *et al.,* 2007).

Further studies proved that LA isomerase-containing microorganisms were capable of successful isomerization of LA to CLA (Lin e*t al.*, 1999). A number of microorganisms were identified as having this capability: namely strains of *Propionibacterium, Lactobacillus, Lactococcus, Streptococcus* and *Bifidobacterium* (Prandini *et al.,* 2007).

Furthermore, it was confirmed that CLA was produced in skim milk, with the addition of LA and *L. acidophilus*. *L. acidophilus* is amongst the most successful amongst LAB strains in converting LA to CLA (Prandini *et al.*, 2007).

In studies by Partanen *et al.* (2001) the influence of FA's, oils and the pH of the medium are some issues affecting microbial CLA synthesis. They found that some unsaturated fatty acids (UFA's) are stronger inhibitors of CLA production than SFA's. The mechanism by which this inhibition takes place is still not quite understood.

Presence of other nutrients

The presence of proteins in the milk or dairy products may also have a major effect on the final CLA concentration (O'Shea *et al.*, 2000). The presence of high quality proteins (acting as hydrogen donors) present in the raw milk, resulted in higher CLA levels (Lin *et al.*, 1999). Shanta & Decker (1993) found that with added proteins, such as caseinate and whey protein, there were increased CLA levels in processed cheese.

Heat treatment of dairy products

Processing has little to no effect on the final CLA level according to a study by Lock & Bauman (2004). In a similar study by Luna *et al.* (2007) different temperatures were evaluated during the manufacturing of cheese and no significant changes were observed in the CLA levels.

Heating using microwaves also negatively influenced the final CLA concentration in cheese (Rodríguez-Alcalá & Fontecha, 2007). Cheese exposed to microwaves for 5 and 10 minutes, showed a significant decrease in CLA of 21 and 53 %. The same was found for skim milk exposed to pasteurization (Rodríguez-Alcalá & Fontecha, 2007). High temperature short time (HTST) pasteurization caused a significant decrease of CLA (Campbell *et al.*, 2003).

Other authors found that pasteurization had a positive effect on the final CLA concentration in dairy products (Campbell *et al.*, 2003; Herzallah *et al.*, 2005). They confirmed that the total CLA content significantly increased for processed cheese when the cheese milk was pasteurized at temperatures between 80 and 90°C (Lin *et al.*, 1995; Campbell *et al.*, 2003; Herzallah *et al.*, 2005). Luna *et al.* (2007) therefore suggested that only temperatures above the traditional processing temperatures can cause a significant decrease of CLA.

Cheese ripening

The ripening time plays an important role in the formation of CLA. Due to the formation of CLA from LA, an increase in CLA levels was found after week four to eight of cheese ripening (Lin *et al.*, 1999). Contrary to this, Luna *et al.* (2007) reported that the CLA levels in Edam cheese were not significantly affected by ripening.

Refrigerated storage of fermented dairy products

No significant changes in the CLA content were found during refrigeration of cheese (Lin *et al.*, 1999; Campbell *et al.*, 2003) and yogurt (Rodríguez-Alcalá & Fontecha, 2007). According to (Xu *et al.*, 2005) the small, but not significant decrease of CLA during storage may be due to the fact that the combination of most probiotic bacteria with yogurt cultures produced slightly higher contents of CLA1 and CLA2 isomers which compensate for the decrease in CLA.

DIETARY RECOMMENDATIONS FOR CONJUGATED LINOLEIC ACID INTAKE

It is estimated that a 70 kg human should consume about 3.0 to 3.5 g of CLA per day, to obtain maximum health benefits (Rodríguez-Alcalá & Fontecha, 2007; Akalin *et* al, 2007; Hur *et al*, 2007). According to the results from a study by Rodríguez-Alcalá & Fontecha

(2007) the average estimated daily CLA intake was in the range of 0.15 g to 1.5 g per day, depending on the gender and age. The recommended dietary allowance (RDA) for CLA is more than three times the daily consumption of the average adult according to the values in the study of Rodríguez-Alcalá & Fontecha (2007); therefore it became necessary to increase the CLA levels in foods (Hur *et al.*, 2007). In a study by Ip *et al.* (1994) they estimated the same values for animals, but suggested that the amount of CLA that a human being should consume is higher. In a study by Parodi (1994) it was suggested that the more realistic achievable amount of CLA to be consumed, should be 500 to 1500 mg/day. This intake should provide humans from one-third up to the entire dose extrapolated to achieve measurable human beneficial effects. According to Ritzenhaler *et al.* (2001) the average intake for men must be 620 mg per day and for women, 441 mg per day for cancer prevention.

CONSUMER PERCEPTIONS REGARDING CONJUGATED LINOLEIC ACID FORTIFICATION OF DAIRY

Consumers increasingly believe that food contributes directly to their health (Young, 2000; Mollet & Rowland, 2002). Food is not intended to only satisfy hunger and to provide necessary nutrients anymore, but also to prevent nutrition-related diseases and improve physical and mental well-being (Roberfroid, 2000b; Menrad, 2003). The increased demand for functional foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy and the desire for older people for improved quality of their later years (Roberfroid, 2000a; Roberfroid 2000b; Kotilainen *et al.*, 2006).

The term "functional food" originated in Japan in the 1980's for food products fortified with special constituents that possess advantageous physiological effects (Hardy, 2000; Kwak & Jukes, 2001; Stanton *et al.*, 2005).
The dairy-based beverages market may be seen as the market of main focus when the sales of yogurt, milk and other beverages are taken into consideration. Dairy beverages containing probiotics and or prebiotics dominate the functional dairy beverage market, but the focus has also moved to CLA enriched dairy products (Gagada *et al.*, 1999).

Before any new CLA products can be marketed, it is important to understand the consumers' perceptions towards such products (Peng *et al.*, 2006). The type of factors that may influence the consumers' attitudes and acceptance, include product quality, attributes

and price. The labeling of the products is also very important, as it will influence the consumers' understanding and acceptability of CLA enriched dairy products (Peng *et al.*, 2006). Research confirmed that consumers are willing to pay for CLA enriched products (Jimenez *et al.*, 2008), especially those with a history of cancer (Campbell *et al.*, 2003). It appears that women and consumers over the age of 25 are those willing to pay more for food products with increased health benefits (Campbell *et al.*, 2003).

METHODS OF CONJUGATED LINOLEIC ACID FORTIFICATION

Bovine diet modification

Milk can be enriched with CLA by modifying the bovine diet. Supplementation of seeds, e.g. rapeseed, sunflower seeds and linseeds in the bovine diet, increases the CLA concentration in the milk produced (Mallia *et al.*, 2008). Addition of SFO and soybean oil to the diet resulted in high CLA production levels. Soybean oil addition to the diet showed the highest levels of CLA, even higher than addition of SFO, which resulted in a 500 % higher CLA level than observed with regular diets (Bell & Kennelly, 2001).

The diets that resulted in the highest level of CLA, also showed the lowest level of SFA's in the milk (Bell & Kennelly, 2001). When synthetic CLA was added to the diet to produce CLA-rich milk, the CLA must be protected from the rumen environment by encapsulation of the CLA with formaldehyde-treated casein (Bell & Kennelly, 2001).

To reach the recommended dietary level of CLA, 30 servings of naturally increased CLA dairy must be consumed; therefore options of direct fortification of dairy must rather be considered (Campbell *et al.*, 2003).

Addition of linoleic acid

Linoleic acid can be added to media to be converted to CLA by LAB in starter cultures. Too high a dosage of LA (> 1 mg/ml) can inhibit the growth of certain starter strains as the LAB are then exposed to a too high concentration of UFA's (Kim & Liu, 2002; Sieber *et al.*, 2004). There is still some controversy whether LA must be in the free acid form or whether LA must be esterified. The esterified form is more stable than the free acid form. There

were studies that proved that both forms are equally effective; other studies found that the free acid form was more effective when used as a supplement to produce CLA via starter cultures (Kim & Liu, 2002). Sunflower oil is an excellent alternative source of LA. *L. lactis* produced the highest amount of CLA compared to other LAB in a study by Sieber *et al.* (2004) with SFO supplementation.

Addition of synthetic CLA to dairy products

Commercial synthetic CLA is available in two forms: as oil and as an encapsulated powder form. A mixture of up to 18 CLA isomers is present in the oil supplement. The predominant isomers are CLA1 and CLA2 (Rodríguez-Alcalá & Fontecha, 2007). There is no structural difference between the naturally existing CLA and the synthetic CLA isomers (Hur *et al.*, 2007).

Microencapsulated CLA is prepared when CLA is added to whey and casein protein concentrate diluted in deionized water which is then spray dried to create CLA encapsulated by a protein capsule. Care should be taken in the amount of the powder added to the yogurt, as a too high amount can lead to a powdery or cardboardy taste, excessive firmness and a grainy texture (Mistry & Hassan, 1992; Guzmán-González *et al.*, 2000).

Specific starter cultures and media

The formation of CLA by common LAB was studied on several occasions and higher levels of CLA were obtained in certain studies. Results from these studies demonstrated that many strains of *Lactobacilli, Lactococci* and *Streptococci* were able to produce CLA from LA in a special medium or in skim or whole milk as a growth medium (Jiang *et al.*, 1998; Lin *et al.*, 1999; Pariza & Yang, 1999; Lin, 2000; Pariza & Yang 2000; Ogawa *et al.*, 2001; Ham *et al.,* 2002; Kim & Liu, 2002; Alonso *et al.,* 2003; Coakley *et al.,* 2003; Kishino *et al.,* 2003; Lin, 2003; Lin *et al.*, 2003; Lin, 2006). The reports in literature are however contradictory, since there are some authors who reported negative results while studying CLA production by certain starter cultures (Lin *et al.,* 2003; Sieber *et al.,* 2004; Lin, 2006).

L. lactis ssp. *cremoris* was a very effective producer of CLA. When LA was added to a skim milk powder medium, *L. lactis* ssp. *cremoris* produced high amounts of CLA (Sieber

et al., 2004). Studies were done on the production of CLA by different starter cultures in a skim milk powder medium with added LA. The effectiveness of the different starter cultures in converting LA to CLA is illustrated in Fig. 2.3. It was evident that *L. acidophilus* was the most effective starter strain at maximum LA addition level of 1 mg /ml media (Sieber *et al.*, 2004).

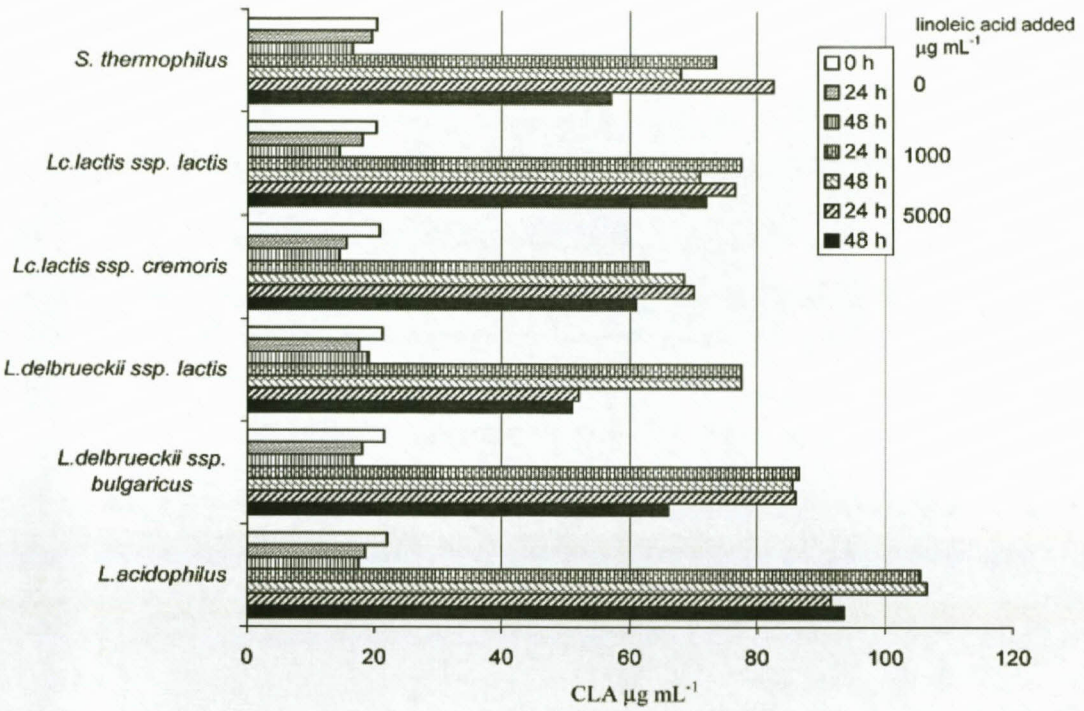


Figure 2.3 ***The ability of lactic acid bacterial strains to convert linoleic acid to conjugated linoleic acid*** (Sieber *et al.*, 2004).

TECHNOLOGICAL EFFECTS OF CONJUGATED LINOLEIC ACID FORTIFICATION

Processing- and storage properties

Conjugated linoleic acid fortified cheese studied by Collomb *et al.* (2006) had a reduced fat globule and casein micelle size as well as an alteration of protein distribution in the casein micelles. This affected the cheese processing properties (Collomb *et al.*, 2006). Cheddar cheese manufactured from milk with a seven-fold higher CLA level than conventional milk, ripened much faster during the first three months of ripening (Collomb *et al.*, 2006).

Texture

Cheddar cheese with elevated CLA levels developed a more desirable texture, as it was softer than non-fortified cheese. The softer texture may be attributed to the higher level of PUFA's in the cheese (Collomb *et al.*, 2006).

Conjugated linoleic acid enriched butter had increased levels of UFA's and lower levels of short and medium chain FA's (Bauman *et al.*, 2000). The CLA enriched butter was also more spreadable than conventional butter and the shelf life at 5 to 6 °C, was also equivalent to that of conventional butter (Mallia *et al.*, 2008).

Oxidation

Milk lipids are major contributing factors in determining the consumer acceptability of most dairy products. The reaction of milk fats with oxygen can result in flavour deterioration due to oxidation and this creates serious problems in the storage stability of dairy products. The occurrence of autoxidation and the degree thereof, depends on the specific product. Lipid oxidation is the reaction of FA's with molecular oxygen (O'Brien & O'Connor, 2002). Lipid oxidation results in the formation of hydro peroxides that can easily react with FA's leading to the formation of secondary oxidation products, essentially aldehydes. This process is dependent on the availability of substrates, however external factors such as light exposure, temperature and the presence of pro-oxidant compounds also play a role (Serra *et al.*, 2008).

In a study where the oxidative stability of CLA in milk and milk products was measured, the CLA milk showed a very low oxidative stability. Secondary oxidation was mainly correlated with aldehydes (Timm-Heinrich *et al.*, 2004). The conjugated double bonds in CLA decrease the oxidative stability of the CLA (Nawar, 1996). Due to the fact that CLA may be easily oxidized, several studies have suggested that it must be protected, e.g. by means of microencapsulation to increase the oxidative stability (Kim *et al.*, 2000; Park *et al.*, 2002; Jimenez *et al.*, 2008).

In another study by Jones *et al.* (2005), it was found that exposure of CLA enriched UHT milk to light, did not seem to exert adverse effects. In general lipolysis and lipid oxidation

do not cause problems in conventionally produced yogurt. This may be due to a combination of factors such as low pH, low storage temperatures, relatively short shelf life and high normal flavour level (Deeth, 2002). There are some authors who reported the chemical and physical characteristics of CLA-enriched cheese, but the data on the triglyceride composition and lipid oxidation status is relatively limited (Jones *et al.*, 2005; Coakley *et al.*, 2007).

Sensory properties

Formation of off-flavours in milk and dairy products may occur due to light-exposure or it may be a result of microbial activity (Marsili, 2002). The main cause of the off-flavours is lipid oxidation and lipolysis (Marsili, 2002). Lipid oxidation results in the formation of odourless and tasteless, but rather unstable hydro peroxides. Other components that can also originate from milk fat oxidation are some ketones and alcohols. Hydro-peroxides can easily react with FA's leading to the formation of secondary oxidation products, essentially aldehydes, resulting in rancid and cardboard tastes of dairy products (Marsili, 2002).

Cheese produced from milk with a three times higher CLA level, showed no sensory difference from cheese made from conventional milk (Collomb *et al.*, 2006). Many studies confirmed that modified milk did not have sensory defects even when the CLA concentration was increased many-fold when compared to conventional milk (Ramaswamy *et al.*, 2001). Other studies reported negative technological effects and the presence of grassy or vegetable flavours in milk (Campbell *et al.*, 2003).

Viscosity

Generally the viscosity of yogurt decreases during the storage period. This is usually due to whey separation, which is the expulsion of the whey from the gel network and leads to spontaneous syneresis. The syneresis can be induced by damage to the gel matrix mainly caused by the activity of the LAB. Yogurt starter cultures, containing LAB such as *L. delbrueckii* spp. *bulgaricus* and *S. thermophilus* are active even at low temperatures and can produce small amounts of lactic acid by the fermentation of lactose, which further results in a pH decrease (Shah *et al.*, 1995). The fact that the acidity may increase during storage leads to the problem of sensitivity of the LAB to the acidity. This post-acidification

during storage is due to the ß-galactosidase (ß-gal) secreted by the LAB, which is still active between 0°C and 5°C, resulting in the formation of D-glucose and D-galactose. The glucose is further fermented by LAB to lactic acid, causing the pH to decrease to less than 4.2 and result in whey separation which also affects the LAB viability due to more hydrogen ions than lactate ions (Rasic & Kurman, 1978).

Syneresis can be minimized by using stabilizers and the most recent approach is to increase the total solids (TS) content (Lucey *et al.*, 1998). The physical properties of the yogurt are greatly influenced by the amount of TS present. With the addition of encapsulated CLA, the viscosity of the yogurt increases due to the increase in the TS content (Guirguis *et al.*, 1984; Becker and Puhan, 1989; Biliarderis *et al.*, 1992; Wacher-Rodarte *et al.*, 1993).

With any attempt to increase the CLA content of a food product, the influence on the products natural properties should first be examined carefully (Wacher-Rodarte *et al.*, 1993).

2.4 CONCLUSIONS

Extensive research on the effect of CLA enriched dairy products on human health has been done (Fogerty *et al.*, 1988; Ha *et al.*, 1989 and Jiang *et al.*, 1998). The estimated daily intake of CLA is far beneath the recommended dose needed to gain maximum health benefits. Although dairy products are the richest natural sources of CLA, the amount present in dairy products, is still too low to make a major contribution to the amount needed to be consumed. There are however, a number of factors that can affect CLA concentration. These factors are processing, storage, ripening, etc. It is important to do thorough research to determine the temperature, pH, etc., at which CLA is relatively stable.

The opportunity of producing CLA fortified products does exist. Introducing high CLA-producing LAB to the products is a strong possibility, as some of these organisms have the ability to convert the LA in the dairy product to CLA. LA can also be added to make more LA available to the LAB for conversion to CLA. Other oils such as SFO may be added to the milk medium as a LA source.

Direct fortification of dairy products with synthetic CLA is another possible way to increase the CLA level in the final product available for consumption. Direct synthetic CLA fortification may be done by adding CLA oil or microencapsulated CLA powder to the product at a given stage of the manufacturing process. The impact of CLA fortification on the physical, chemical and sensory properties of the specific product must be evaluated.

A major concern is that the enhancement of the CLA concentration in dairy products might have a negative impact on production costs, but due to the health benefits associated with a CLA fortified product, consumers will probably be willing to accept the increase in price.

CHAPTER 3

BIOCONVERSION OF LINOLEIC ACID FROM EXTERNAL LIPID SOURCES TO CONJUGATED LINOLEIC ACID BY YOGURT STARTER CULTURES

ABSTRACT

The aim of this study was to increase the conjugated linoleic acid content of yogurt. Three yogurt starter cultures (YC-180, YC-X11 and ABT-5) were evaluated for their ability to convert linoleic acid to conjugated linoleic acid, with linoleic acid or sunflower oil supplementation, in fat free or full cream yogurt. Supplementation with linoleic acid and sunflower oil did not significantly affect the total fat content of the yogurt, but altered the fatty acid profiles of the yogurt. Differences in the conjugated linoleic acid concentrations of the yogurt made from the milk inoculated with the three different starter cultures occurred. The highest conjugated linoleic acid concentration obtained in the yogurt made from skim milk and the YC-180 culture, was approximately 0.45 mg/100 g yogurt with the addition of linoleic acid. The highest conjugated linoleic acid concentration obtained in the yogurt made from full cream milk and the YC-X11 culture, was approximately 34.5 mg/100 g yogurt with the addition of sunflower oil. The type of linoleic acid source, milk fat content and the type of starter culture used, are factors that influenced the final conjugated linoleic acid concentration of the yogurt.

Keywords: Conjugated linoleic acid; linoleic acid; sunflower oil; starter cultures

3.1 INTRODUCTION

Conjugated linoleic acid (CLA) is a group of polyunsaturated fatty acids (PUFA's) and is a product of linoleic acid (LA) isomerization. Conjugated linoleic acid isomers have been recognized as anti-oxidants, cancer inhibitors, cholesterol depressing agents and growth-promoting factors. Of all the existing CLA isomers, the C18:2c9,t11 (CLA1) and

C18:2t10c12 (CLA2) isomers are the most biologically active (Ha *et al.*, 1987, 1989; Ip *et al.,* 1991; Schultz *et al.*, 1992). In several studies (Schultz *et al.*, 1992; Ip *et al.*, 1994; Scimeca, 1999) it was found that CLA was also effective in the prevention and treatment of breast cancer, malignant melanoma, colorectal cancer, leukemia, prostate cancer and ovarian cancer.

Ruminant dairy products are the richest natural sources of CLA (Ha *et al.*, 1989; Chin *et al.*, 1992; Shanta *et al.*, 1995). Conjugated linoleic acid isomers in dairy products are formed through the isomerization of LA by the isomerase enzymes which are present in the rumen bacteria or through the oxidation of LA during processing (Kepler & Tove, 1967; Christie, 1983; Ha *et al.*, 1987, 1989; Chin *et al.*, 1992; Chin *et al.*, 1993).

The CLA content of dairy products may vary significantly. Raw milk contains approximately 0.83 to 5.5 mg CLA/g fat (Ha *et al.*, 1989). The average level of CLA in dairy products is usually in the range of 0.55 to 9.12 mg CLA/g fat, depending on the specific product (Lin & Lee, 1997). Brick cheese contains CLA at levels as high as 7.1 mg CLA/g fat and non-fat yogurt contains CLA at levels in the range of 1.7 to 5.3 mg CLA/g fat (Ha *et al.*, 1989; Chin *et al.*, 1992; Shanta *et al.*, 1995). In a study by Shanta *et al.* (1995) they discovered that fermented dairy products in general contain higher levels of CLA than non-fermented dairy products. A suggested explanation for the higher CLA levels in fermented milk products was that some lactic acid bacteria (LAB) have the ability to produce CLA (Shanta *et al.*, 1995).

Some authors studied the production of CLA by LAB and identified some strains of *Lactobacilli, Lactococci* and *Streptococci* that were able to produce CLA in a milk medium (Jiang *et al.*, 1998; Lin *et al.*, 1999; Pariza & Yang, 1999; Lin, 2000; Ogawa *et al.*, 2001; Ham *et al.,* 2002; Kim & Liu, 2002; Alonso *et al.,* 2003; Coakley *et al.,* 2003; Kishino *et al.,* 2003; Lin, 2003; Lin *et al.*, 2003; Lin, 2006).
In another study (Lin *et al.*, 1999), no significant differences occurred between fermented milk samples that contained different LAB. This was attributed to the exhaustion of the available LA for CLA conversion, which indicated the need of LA addition for CLA formation. Other authors also suggested that a LA source should be added to the milk medium as the amount of LA in milk available to the LAB for conversion to CLA is limited (Jiang *et al.*, 1998; Lin *et al.*, 1999; Pariza & Yang, 1999; Lin, 2000; Ogawa *et al.*, 2001;

Ham *et al.,* 2002; Kim & Liu, 2002; Alonso *et al.,* 2003; Coakley *et al.,* 2003; Kishino *et al.,* 2003; Lin, 2003; Lin *et al.*, 2003; Lin, 2006).

For many years yogurt has been seen as a food product that contributes positively to human health (Tamime & Robinson, 1991; Bertolami, 1999; Shah, 2001; Sloan, 2000; Milo-Ohr, 2002). It was therefore decided to use yoghurt as a model for this research. The aim of this experiment was to increase the CLA concentration of yogurt with the addition of LA sources and with the use of specific starter cultures.

3.2 MATERIALS AND METHODS

STARTER CULTURES

Three of the most commonly used starter cultures in the South African dairy industry were evaluated for their ability to increase the CLA concentration of yogurt. Starter cultures were supplied by CHR-Hansen, South Africa.

The YC-180 and YC-X11 cultures were both from the Yo-Flex® range of yogurt starter cultures. The ABT-5 culture was from the Probio-Tec® range of probiotic cultures that can be used in yogurt fermentation.

The three starter cultures that were used with the LAB strains contained in each starter culture mixture are listed in Table 3.1.

Table 3.1: Description of starter cultures used in this study.

Code	Strains
YC-180 - Yo-Flex®	*Streptococcus salivarius* spp. *thermophilus*, *Lactobacillus delbrueckii* spp. *lactis* and *Lactobacillus delbrueckii* spp. *bulgaricus*
YC-X11 – Yo-Flex®	*Streptococcus salivarius* spp. *thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*
ABT-5 – Probio-Tec®	*Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12 and *Streptococcus salivarius* spp. *thermophilus*

EXTERNAL LIPID SOURCES

In this study LA supplied by Merck (South Africa), was used as a source of LA. This product was 90 % pure. Nola sunflower oil (SFO) supplied by Chipkins (South Africa) was used as a cheaper alternative for LA. The SFO contained 67.4 % pure LA.

YOGURT MANUFACTURE

Full cream milk powder and skim milk powder from Parmalat (South Africa) were rehydrated at 50°C for one hour. A concentration of 2.5 % sucrose (Huletts, South Africa) was added after rehydration. The rehydrated full cream milk and rehydrated skim milk were pasteurized at 90°C for 8 to 10 minutes. Pasteurized milk (100 ml) was then added to each of 108 flasks (250 ml each) in which the fermentation took place. The milk was allowed to cool down to 42°C. The milk was then inoculated with the appropriate starter culture and maintained at 42°C until a pH of 4.6, the iso-electric point of casein proteins, was reached. The yogurt was then rapidly cooled down to 4°C to stop the fermentation process (Tamime & Robinson, 1996; Vandewegh *et al.*, 2002; Arkbage, 2003).

With each of the three yogurt starter cultures, full cream yogurt and fat free yogurt control samples, full cream and fat free yogurt samples with LA and full cream and fat free yogurt samples with SFO were prepared with six replicates of each (Table 3.2).

Table 3.2: Lay-out of the yogurt manufacturing process.

Sample description	**Number of yogurt batches prepared**		
	YC-180	**YC-X11**	**ABT-5**
Full Cream + ~263.16 mg LA	6	6	6
Full Cream + ~370.9 mg SFO	6	6	6
Full Cream Control	6	6	6
Fat Free + ~263.16 mg LA	6	6	6
Fat Free + ~370.9 mg SFO	6	6	6
Fat Free Control	6	6	6

ADDITION OF EXTERNAL LIPID SOURCES

An amount of 263.16 mg LA and 370.9 mg SFO was added to the appropriate 250 ml batches to supply a LA concentration of 1 mg/ml LA, which was recommended in literature (Sieber *et al.*, 2004; Kim & Liu, 2002). Higher concentrations of LA have an inhibiting effect on the growth and metabolism of the lactic acid starter bacteria (LAB) (Sieber *et al.*, 2004; Kim & Liu, 2002).

SUSPENSION OF EXTERNAL LIPID SOURCES IN MILK

All yogurt samples, including the control samples, were homogenized with an Ultra Turrax T-25 (Janke & Kunkel IKA-Labortechnik) at 8000 rpm for 1 min to ensure that the LA and SFO were evenly distributed for maximum exposure to the starter culture bacteria in the milk medium.

LIPID EXTRACTION

Total lipid from the 108 yogurt samples were quantitatively extracted, according to the method of Folch *et al.* (1957) using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001 % to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under vacuum and the extracts were also dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as moisture adsorbent.

Total extractable fat content (TEFC) was determined gravimetrically and expressed as % fat (w/w) per 100 g yogurt. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % total solids (TS) (w/w) per 100 g yogurt.

The moisture content of the yogurt was determined by subtraction (100% - % lipid - % TS) and expressed as % moisture (w/w) per 100 g of yogurt. The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C until further analyzed.

FATTY ACID ANALYSIS

The lipid (from Folch extraction) was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acids were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Park & Goins, 1994). Conjugated linoleic acid isomers were quantitatively determined by using heptadecanoic acid (C17:0) as internal standard. The CLA1 and CLA2 could then be expressed as mg CLA/g fat. The areas of the CLA isomers were expressed against the area of the internal standard. Correction factors for different CLA isomers were also calculated. Peak identification of the fatty acids and the fatty acid profile were done with an external standard, Supelco 37 component FAME Mix.

Fatty acid methyl esters (FAME) were quantified using a Varian GX 3400 flame ionization Gas Chromatograph (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 μm film thickness). The column temperature was 40 to 230°C (hold 2 min; 4°C/ min; hold 10 min).
FAME in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the chromatograms. FAME samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19).

Fatty acids (FA's) were expressed as the relative percentage of each individual FA as a percentage of the total of all FA's present in the sample and the CLA could be expressed as mg CLA isomer/100 g of yogurt.

STATISTICAL ANALYSIS

This experiment was a 2 x 2 x 3 factorial design representing the two fat levels, two external lipid sources and the three starter cultures with six replicates per treatment.

An analysis of variance (ANOVA) procedure for balanced data (NCSS, 2007) was used to determine the effect of SFO inclusion and LA inclusion and their interaction with specific starter cultures, on the LAB in the yogurt starter cultures, fatty acid composition, fatty acid

ratios and actual CLA content of yoghurt. A one way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine whether the above mentioned variables were significantly influenced by the two lipid sources. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

3.3 RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for the effect of the initial fat content of the milk, the lipid source and the type of starter culture that was used as well as the interactions of these on the proximate analysis, the FA composition, FA ratios and the actual CLA content of the final yogurt product is shown in Table 3.3. Fat content and lipid source had a significant ($p < 0.001$) effect on proximate composition, most FA's and all FA ratios. Only fat content, TS content, C4:0 content, C13:0, C18:1 content, CLA1 content, C24:0, C24:1c15 (C24:1) and MUFA content were significantly ($p < 0.05$) influenced by the culture type.
Only a few parameters were significantly influenced by the interactions between fat content, lipid source and culture type. These interactions will be discussed with the main effects. The actual CLA content of the yogurt was significantly ($p < 0.001$) influenced by the fat content and culture type. Lipid source had a statistically significant ($p < 0.001$) effect on the CLA2 content. All the interactions between fat content, lipid source and culture type had a significant ($p < 0.001$) effect on actual CLA content.

PROXIMATE COMPOSITION

Milk fat globules (MFG) in dairy products contribute to creaminess and together with its own flavour serves as the main compound for many flavour developments (Frøst & Janhøj, 2007). When the milk fat percentage is low, crystals become irregular in shape and this can affect the texture of the product (Tietz & Hartel, 2000). MFG can act as structure breakers in gelled dairy products. Lower creaminess in some dairy products is due to the lower fat content in the product (Frøst & Janhøj, 2007).

The influence of starter culture, LA addition and SFO addition on the total fat content of the full cream yogurt and the fat free yogurt is illustrated in Fig. 3.1. The fat content of the milk used during the yogurt manufacturing significantly ($p < 0.001$) influenced the total fat content of the final yogurt sample. It was expected that the full cream yogurt would have a

much higher total fat content than the fat free yogurt. The full cream yogurt contained approximately the same amount of total fat which was around 3 %, as the full cream milk from which it was made. The same trend was observed with the fat free yogurt, but only at a lower level, since fat free milk usually has a fat percentage lower than 0.5 % (Belitz & Grosch, 2008).

Significant differences ($p < 0.001$) were observed in fat content of yogurt manufactured with different lipid sources (Fig. 3.1). The control yogurt samples of both the fat free yogurt and the full cream yogurt had a slightly lower total fat content than the full cream yogurt and fat free yogurt supplemented with LA and SFO. For both the full cream yogurt and the fat free yogurt, the samples that were supplemented with SFO had higher total fat content than the yogurt samples that were supplemented with LA (Fig. 3.1). It may be attributed to the fact that more SFO was added than LA in order to supply the same final concentration of 1 mg LA/ml to the yogurt starter cultures, because the SFO had a lower LA concentration than the LA that was used (see section 3.2). According to studies by Kim & Liu (2002) and Sieber *et al.* (2004) a higher LA concentration can inhibit the growth and metabolism of the starter culture bacteria.

The same results were found by Urbach (1995) who suggested that some of the fat was broken down into less complex components which are then metabolized by the LAB in the starter cultures. These processes of fat breakdown also contributed to the development of the characteristic flavours in the yogurt (Urbach, 1995).

In Fig. 3.2 a significant ($p < 0.001$) difference can be observed between the total moisture content of the full cream yogurt and the total moisture content of the fat free yogurt. The average total moisture content of all the full cream yogurt samples was significantly ($p < 0.001$) lower than the average total moisture content of the fat free yogurt samples. There is an inverse relationship between the total fat content and the total moisture content of the yogurt. These results were in agreement with the findings of Bonczar *et al.* (2002) and Belitz & Grosch (2008) who also found that milk products with a higher fat content contained lower levels of total moisture.

A lower TS content was observed for the full cream yogurt compared to the fat free yogurt (Fig. 3.2). It is expected that yogurt with a higher total fat content (Fig. 3.1) also has a lower TS content and moisture content (Bonczar *et al.*, 2002; Belitz& Grosch, 2008).

In the case of the full cream yogurt and fat free yogurt, the control yogurt samples had slightly but not significantly higher TS content than the yogurt supplemented with LA and SFO (Fig. 3.2). The higher TS content of the control yogurt samples was due to the lower total fat content obtained for the yogurt samples supplemented with LA and SFO (Belitz & Grosch, 2008).

The results in Fig. 3.2 showed that a difference in the TS content of the yogurt samples manufactured with the three different yogurt starter cultures occurred. For all the lipid treatments the yogurt manufactured with the ABT-5 starter culture had slightly lower TS content than the yogurt manufactured with the YC-180 and YC-X11 starter cultures (Fig. 3.2). This can be attributed to variations in the metabolic activities of the LAB in the different starter cultures. Some of the solids might have been metabolized by the LAB. The degree of the breakdown of the solids as energy source depended on the strain and combination of LAB in the starter cultures (Ozer *et al.*, 1998).

FATTY ACID ANALYSIS

Fatty acid profile

The total amount of fat, individual FA's and the FA ratios play a major role in the texture characteristics, sensory properties, product stability and health aspects of a dairy product (Marsili, 2002; Timm-Heinrich *et al.*, 2004; Collomb *et al.*, 2006).

Table 3.4 indicates that the full cream yogurt samples contained significantly ($p < 0.001$) higher concentrations of the short chain saturated fatty acids (SFA's) than the fat free yogurt samples. The higher levels of SFA's obtained for the full cream yogurt were due to the higher concentration of the natural milk fat present in the full cream yogurt (Chouinard *et al.*, 1999). The full cream yogurt control samples had higher concentrations of the short chain SFA's than the full cream yogurt samples with added LA and SFO (Table 3.4).

The same trend was observed for the fat free yogurt samples. Chouinard *et al.* (1999) also found that increased levels of unsaturated fatty acids (UFA's) resulted in lower levels of short and medium chain SFA's. Inclusion of SFO and LA to the yogurt resulted in a

Table 3.3 : Analysis of variance (ANOVA) on proximate composition, fatty acid composition, fatty acid ratios and actual conjugated linoleic acid content for the effect of fat content, lipid source and culture type.

	Fat Content	Lipid Source	Culture Type	Fat content X Lipid Source	Fat Content X Culture Type	Lipid Source X Culture Type	Fat Content X Lipid Source X Culture Type
Proximate analysis:							
Fat content (%)	***	***	**	**	*	NS	NS
Moisture content (%)	***	***	NS	NS	NS	NS	NS
Solids content (%)	***	*	*	NS	NS	NS	NS
Fatty Acid Composition (%):							
C4:0	***	***	**	***	*	*	**
C6:0	***	***	NS	***	NS	NS	NS
C8:0	***	***	NS	***	NS	NS	NS
C10:0	***	***	NS	***	NS	NS	NS
C12:0	***	***	NS	***	**	NS	NS
C13:0	***	**	*	**	*	NS	NS
C14:0	***	***	NS	***	**	*	*
C14:1c9	***	***	NS	***	NS	NS	NS
C15:0	***	***	NS	***	NS	NS	NS
C16:0	***	***	NS	***	NS	*	*
C16:1c9	***	***	NS	***	NS	NS	*
C17:0	***	***	NS	***	NS	*	NS
C17:1c10	***	***	NS	***	NS	*	*
C18:0	***	***	NS	***	NS	NS	NS
C18:1t9	**	***	NS	***	NS	NS	NS
C18:1c9	***	***	*	***	**	*	NS
C18:2t9,12 (n-6)	***	NS	NS	NS	NS	NS	NS
C18:2c9,12 (n-6)	***	***	NS	***	NS	NS	NS
C18:3c9,12,15 (n-3)	NS	NS	NS	NS	NS	NS	NS
C18:2c9,t11 (n-6) (CLA)	***	***	**	***	***	***	***
C18:2t10,c12 (n-6) (CLA)	***	***	NS	***	NS	***	**
C20:0	***	***	NS	***	NS	*	NS
C20:1c11	***	NS	NS	NS	NS	**	NS
C20:3c11,14,17 (n-3)	***	*	NS	*	NS	NS	NS
C20:3c8,11,14 (n-6)	NS	NS	NS	NS	NS	NS	NS
C20:4c5,8,11,14(n-6)	***	NS	NS	NS	NS	NS	NS
C20:5c5,8,11,14,17 (n-3)	***	***	NS	**	NS	**	*
C21:0	NS	NS	NS	NS	NS	NS	NS
C22:0	***	***	NS	***	NS	NS	NS
C24:0	*	***	*	***	*	***	*
C24:1c15	***	***	*	***	NS	*	*
Fatty Acid Ratios (%):							
SFA	***	***	NS	***	*	NS	NS
MUFA	***	***	*	***	*	***	**
PUFA	***	***	NS	***	NS	NS	NS
n-3	**	**	NS	**	NS	**	*
n-6	***	***	NS	***	NS	NS	NS
Actual conjugated linoleic acid content:							
C18:2c9,t11 (n-6) (CLA) (mg/g fat)	***	NS	***	***	***	***	***
C18:2t10,c12 (n-6) (CLA) (mg/g fat)	***	***	***	***	***	***	***
mg Total Conjugated Linoleic Acid/100g yoghurt	***	NS	***	NS	***	***	***

NS = Not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

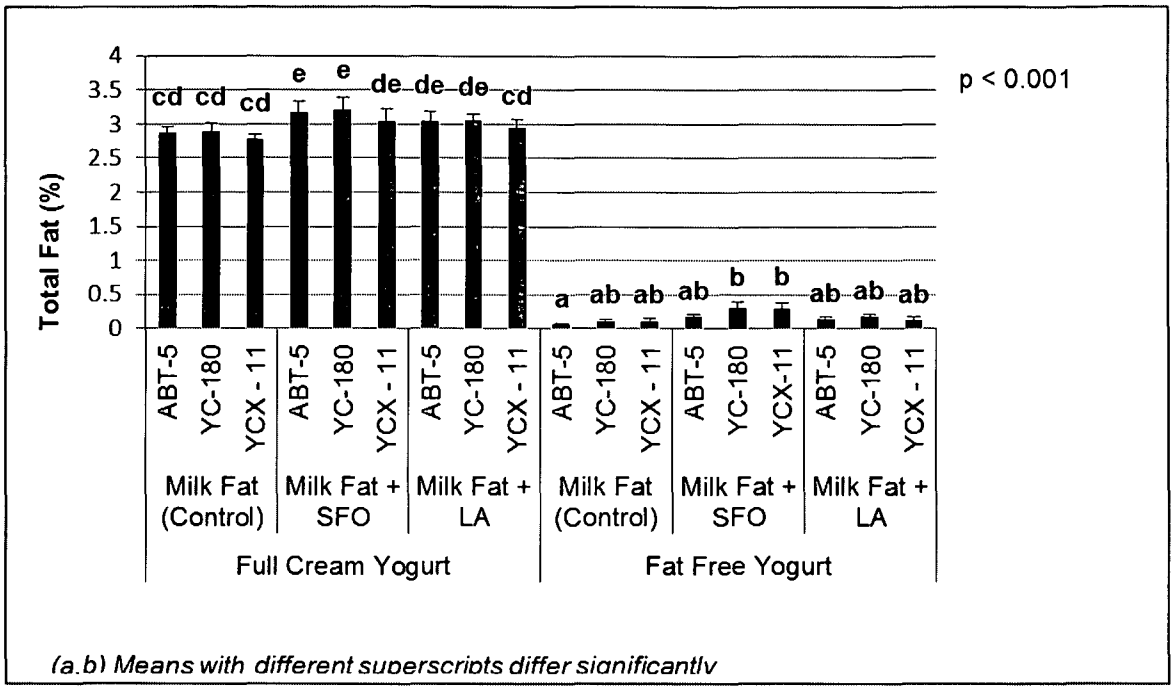


Figure 3.1 Influence of the different treatments on the final total fat content of the yogurt. Means with different superscripts differ significantly.

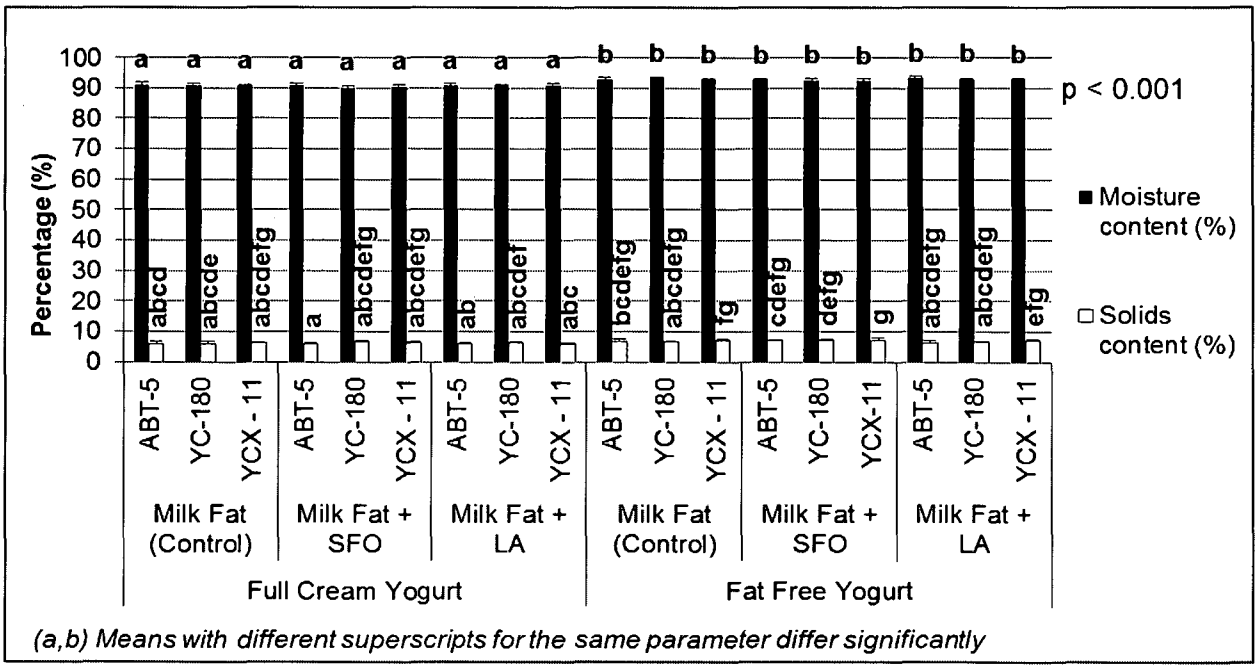


Figure 3.2 Influence of the different treatments on the total moisture content and total solids content of the yogurt

Table 3.4: The effect of fat content, lipid source and culture type on the short chain saturated fatty acid content (%) of yogurt.

Fat Content	Lipid source	Culture Type	C4:0	C6:0	C8:0	C10:0	C12:0
Full Cream Yogurt	Milk Fat (Control)	ABT-5	1.44 ± 0.14^{d}	1.96 ± 0.10^{e}	1.50 ± 0.04^{d}	3.92 ± 0.08^{d}	4.54 ± 0.09^{f}
		YC-180	1.43 ± 0.04^{d}	1.95 ± 0.04^{e}	1.50 ± 0.03^{d}	3.94 ± 0.07^{d}	4.60 ± 0.08^{f}
		YCX-11	1.44 ± 0.08^{d}	1.97 ± 0.07^{e}	1.51 ± 0.06^{d}	3.97 ± 0.13^{d}	4.65 ± 0.13^{f}
	Milk Fat + SFO	ABT-5	1.34 ± 0.20^{cd}	1.79 ± 0.14^{e}	1.36 ± 0.07^{d}	3.56 ± 0.17^{d}	4.15 ± 0.18^{de}
		YC-180	1.35 ± 0.05^{cd}	1.84 ± 0.08^{e}	1.40 ± 0.07^{d}	3.70 ± 0.17^{d}	4.31 ± 0.14^{ef}
		YCX-11	1.43 ± 0.10^{d}	1.91 ± 0.12^{e}	1.45 ± 0.08^{d}	3.77 ± 0.17^{d}	4.38 ± 0.18^{ef}
	Milk Fat + LA	ABT-5	1.26 ± 0.20^{bcd}	1.77 ± 0.21^{e}	1.38 ± 0.13^{d}	3.69 ± 0.29^{d}	4.36 ± 0.23^{ef}
		YC-180	1.25 ± 0.26^{bcd}	1.81 ± 0.18^{e}	1.43 ± 0.08^{d}	3.78 ± 0.08^{d}	4.40 ± 0.05^{ef}
		YCX-11	1.41 ± 0.04^{d}	1.91 ± 0.04^{e}	1.46 ± 0.03^{d}	3.84 ± 0.05^{d}	4.49 ± 0.06^{ef}
Fat Free Yogurt	Milk Fat (Control)	ABT-5	0.98 ± 0.53^{bc}	0.93 ± 0.47^{d}	0.49 ± 0.25^{bc}	2.15 ± 0.53^{c}	3.75 ± 0.22^{c}
		YC-180	0.34 ± 0.39^{a}	0.64 ± 0.50^{bcd}	0.29 ± 0.32^{abc}	2.23 ± 0.19^{c}	3.83 ± 0.10^{cd}
		YCX-11	0.93 ± 0.11^{b}	0.84 ± 0.44^{cd}	0.56 ± 0.30^{c}	2.19 ± 0.30^{c}	3.59 ± 0.33^{c}
	Milk Fat + SFO	ABT-5	0.13 ± 0.05^{a}	0.12 ± 0.06^{a}	0.07 ± 0.02^{a}	0.34 ± 0.09^{ab}	0.55 ± 0.12^{a}
		YC-180	0.01 ± 0.03^{a}	0.05 ± 0.06^{a}	0.03 ± 0.03^{a}	0.20 ± 0.10^{a}	0.35 ± 0.13^{a}
		YCX-11	0.06 ± 0.08^{a}	0.04 ± 0.09^{a}	0.03 ± 0.06^{a}	0.15 ± 0.20^{a}	0.34 ± 0.29^{a}
	Milk Fat + LA	ABT-5	0.26 ± 0.06^{a}	0.33 ± 0.10^{ab}	0.23 ± 0.07^{ab}	0.72 ± 0.13^{b}	1.11 ± 0.19^{b}
		YC-180	0.24 ± 0.06^{a}	0.40 ± 0.11^{abc}	0.30 ± 0.07^{abc}	0.74 ± 0.15^{b}	1.13 ± 0.25^{b}
		YCX-11	0.15 ± 0.13^{a}	0.30 ± 0.07^{ab}	0.22 ± 0.05^{ab}	0.65 ± 0.11^{b}	1.05 ± 0.17^{b}
Significance level			$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

(a,b) Means with different superscripts in the same column differ significantly

decrease in the concentrations of the short chain SFA's (C4:0-C12:0) in both full cream and fat free yogurt (Table 3.4). This was also in agreement with the findings by Mohamed *et al.* (1988), Kim *et al.* (1993), AbuGhazaleh & Holmes (2007), AbuGhazaleh *et al.* (2002) and Rego *et al.* (2005).

This decrease in the SFA's may be seen as an improvement of the yogurt FA profile, as the SFA's have been reported by Ney (1991) to contribute to hypercholesterolemic effects. The only effect of starter culture was observed for yogurt manufactured from skim milk where yogurt manufactured with starter culture YC-180 had a significantly ($p < 0.001$) lower C4:0 content compared to yogurt manufactured from starter cultures ABT-5 and YC-X11.

The concentrations for the medium chain and long chain SFA's in the full cream yogurt samples were generally significantly ($p < 0.001$) higher than the concentrations of the medium chain and long chain SFA's found in the fat free yogurt samples (Table 3.5). An exception was the concentration of the C21:0 FA which was not influenced by the fat level of the yogurt (Table 3.5). Another exception was the C16:0 content of control yogurt manufactured from skim milk which had a C16:0 content similar to yogurt manufactured from full cream milk (Table 3.5).

The C18:0 content of the control yogurt manufactured from skim milk was also significantly ($p < 0.001$) higher than that of yogurt manufactured from full cream milk. The higher levels of the medium chain and long chain SFA's obtained for the full cream milk were due to the higher concentration of the natural milk fat present in the full cream yogurt, as milk fat generally contains high levels of SFA's (Chouinard *et al.*, 1999).

The yogurt control samples contained higher levels of the medium chain and long chain SFA's (Table 3.5). No PUFA's were added to the control yogurt samples that could have influenced the SFA concentration. The high concentration of SFA's that was found in the control yogurt samples was due to the SFA's naturally contained in the milk fat (Chilliard *et al.*, 1991; Boylston & Beitz, 2002). This is reflected in the results for the control yogurt samples in Table 3.5. The yogurt samples with added SFO had the lowest concentration of medium and long chain SFA's. The LA and SFO had high concentrations of PUFA's and the addition of the SFO and LA caused the lower SFA concentration in the yogurt with added LA and SFO (Table 3.5). More SFO was added to the yogurt than LA, thus more PUFA's were added, which resulted in the lower SFA content of the yogurt with SFO, compared to the yogurt with LA (Table 3.5). Similar results were obtained by Chilliard *et al.* (1991), Jenkins (1993) and Boylston & Beitz (2002) who also suggested that supplementation of PUFA's had a direct influence on the SFA's content of yogurt.

The full cream yogurt had significantly ($p < 0.001$) lower levels of the C17:1c10 (C17:1) MUFA than the fat free yogurt (Table 3.6). The same trend was observed with C18:1t9 and C18:1c9 with the exception of the fat free yogurt with SFO and fat free yogurt with LA. The fat free yogurt manufactured with SFO had low levels where the fat free yogurt manufactured with LA had higher C18:1t9 levels compared to yogurt manufactured from full cream milk (Table 3.6).

As far as the C18:1c9 content is concerned; the fat free yogurt control and the fat free yogurt with SFO had a higher C18:1c9 content than the full cream yogurt (Table 3.6). This may be attributed to the naturally high C18:1c9 content of SFO (AbuGhazaleh & Holmes, 2007). The fat free yogurt with LA had a lower C18:1c9 content than all the other treatments. This is due to the high C18:2c9,12 (C18:2) content of this treatment (Table 3.6).

The fat free yogurt had a lower concentration of SFA's due to the lower total fat content which explains why the MUFA concentration appeared to be higher in the fat free yogurt samples (Chilliard *et al.*, 1991; Boylston & Beitz, 2002). Three significant starter culture effects were observed. The C16:1c9 (C16:1) content of the full cream control yogurt manufactured from the YC-X11 starter culture was significantly ($p < 0.001$) lower than the yogurt manufactured from the starter cultures ABT-5 (Table 3.6). The C18:1c9 content of the fat free yogurt control manufactured from culture YC-180 was significantly ($p < 0.001$) lower than that of the fat free yogurt control manufactured with starter cultures ABT-5 and YC-X11 (Table 3.6). The C24:1 content of the fat free yogurt with LA manufactured from culture YC-X11 was significantly ($p < 0.001$) lower than that of the fat free yogurt manufactured from starter cultures ABT-5 and YC-180 (Table 3.6).

Table 3.7 indicates that the C18:2t9,12; C20:3c11,14,17 (C20:3) and C20:4c5,8,11,14 (C20:4) levels of the full cream yogurt were significantly ($p < 0.001$) higher than the levels of the same FA's of the fat free yogurt. The C18:2 and C20:5 content of the full cream yogurt were significantly ($p < 0.001$) lower compared to the fat free yogurt for the same lipid treatments. No significant differences were observed in the C18:3c9,12,15 and C20:3c8,11,14 content between the fat free and full cream yogurt. The levels of both the CLA isomers (CLA1 and CLA2) were significantly ($p < 0.001$) higher in the full cream yogurt samples than in the fat free yogurt.

The higher concentrations of the LA (C18:2) that were present in the fat free yogurt, can be attributed to the lower total fat content of the fat free yogurt which also resulted in lower SFA concentrations and therefore higher PUFA concentrations (Chilliard *et al.*, 1991; Boylston & Beitz, 2002). The SFO that was added, as well as the LA that was added, contained very high amounts of LA and because of the low fat levels in the fat free yogurts, the influence of the additional LA was much stronger on the fat free yogurt samples than on the full cream yogurt samples (Casper *et al.*, 1988).

The full cream yogurt samples with LA and SFO added had significantly ($p < 0.001$) higher concentrations of LA (C18:2) than the full cream yogurt control samples (Table 3.7). The fat free yogurt with LA and SFO also had significantly ($p < 0.001$) higher levels of LA than the fat free control yogurt (Table 3.7). The high levels of LA that were detected in the yogurt with SFO and LA were due to the fact that LA accounted for approximately 60 % of the SFO and 90 % of the LA product that were used (Casper *et al.*, 1988).

Table 3.5: The effect of fat content, lipid source and culture type on the medium and long chain saturated fatty acid content (%) of yogurt.

Fat Content	Lipid source	Culture Type	C13:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C24:0
Full Cream Yogurt	Milk Fat (Control)	ABT-5	0.08 ± 0.01^{bc}	14.86 ± 0.31^{fg}	1.34 ± 0.02^{c}	34.18 ± 0.41^{bc}	0.57 ± 0.01^{c}	11.64 ± 0.23^{c}	0.16 ± 0.01^{d}	0.01 ± 0.02	0.04 ± 0.01^{bcde}	0.02 ± 0.02^{a}
		YC-180	0.08 ± 0.01^{bc}	15.07 ± 0.12^{fg}	1.35 ± 0.01^{c}	34.27 ± 0.08^{c}	0.55 ± 0.01^{c}	11.42 ± 0.09^{c}	0.15 ± 0.01^{d}	0.01 ± 0.02	0.04 ± 0.01^{bcde}	0.01 ± 0.02^{a}
		YCX – 11	0.09 ± 0.01^{c}	15.21 ± 0.30^{g}	1.36 ± 0.03^{c}	34.26 ± 0.38^{c}	0.56 ± 0.01^{c}	11.37 ± 0.30^{c}	0.15 ± 0.01^{d}	0.02 ± 0.02	0.04 ± 0.01^{abcd}	0.03 ± 0.01^{a}
	Milk Fat + SFO	ABT-5	0.07 ± 0.02^{b}	13.65 ± 0.43^{de}	1.23 ± 0.03^{c}	31.85 ± 0.51^{b}	0.51 ± 0.01^{c}	10.81 ± 0.31^{c}	0.16 ± 0.01^{d}	0.01 ± 0.01	0.08 ± 0.01^{e}	0.02 ± 0.01^{a}
		YC-180	0.07 ± 0.01^{b}	14.11 ± 0.30^{ef}	1.27 ± 0.03^{c}	32.53 ± 0.52^{bc}	0.52 ± 0.01^{c}	10.99 ± 0.23^{c}	0.16 ± 0.01^{d}	0.01 ± 0.01	0.07 ± 0.01^{de}	0.01 ± 0.01^{a}
		YCX - 11	0.08 ± 0.01^{b}	14.26 ± 0.45^{efg}	1.28 ± 0.04^{c}	32.77 ± 0.57^{bc}	0.53 ± 0.01^{c}	11.14 ± 0.20^{c}	0.16 ± 0.01^{d}	0.01 ± 0.01	0.07 ± 0.01^{de}	0.02 ± 0.01^{a}
	Milk Fat + LA	ABT-5	0.08 ± 0.01^{bc}	14.42 ± 0.43^{efg}	1.29 ± 0.03^{c}	32.83 ± 0.36^{bc}	0.54 ± 0.01^{c}	10.97 ± 0.26^{c}	0.15 ± 0.01^{d}	0.01 ± 0.01	0.04 ± 0.01^{bcd}	0.03 ± 0.01^{a}
		YC-180	0.07 ± 0.02^{b}	14.34 ± 0.09^{efg}	1.28 ± 0.01^{c}	32.84 ± 0.41^{bc}	0.55 ± 0.02^{c}	11.22 ± 0.13^{c}	0.16 ± 0.01^{d}	0.01 ± 0.01	0.05 ± 0.01^{cde}	0.03 ± 0.01^{a}
		YCX - 11	0.08 ± 0.01^{bc}	14.62 ± 0.17^{efg}	1.30 ± 0.02^{c}	32.85 ± 0.29^{bc}	0.54 ± 0.01^{c}	10.93 ± 0.20^{c}	0.15 ± 0.01^{d}	0.01 ± 0.01	0.04 ± 0.01^{abcd}	0.02 ± 0.02^{a}
Fat Free Yogurt	Milk Fat (Control)	ABT-5	0.01 ± 0.01^{a}	12.98 ± 0.49^{cd}	0.80 ± 0.39^{b}	34.60 ± 2.02^{c}	0.26 ± 0.14^{b}	15.24 ± 0.85^{d}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YC-180	0.01 ± 0.01^{a}	13.71 ± 0.42^{de}	0.84 ± 0.16^{b}	37.04 ± 1.32^{d}	0.15 ± 0.16^{ab}	15.75 ± 0.73^{d}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YCX - 11	0.01 ± 0.01^{a}	12.30 ± 0.77^{c}	0.92 ± 0.12^{b}	33.97 ± 1.03^{bc}	0.30 ± 0.15^{b}	15.38 ± 1.33^{d}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
	Milk Fat + SFO	ABT-5	0.01 ± 0.01^{a}	1.92 ± 0.44^{a}	0.15 ± 0.03^{a}	11.00 ± 1.05^{a}	0.08 ± 0.01^{a}	6.05 ± 0.27^{b}	0.25 ± 0.02^{e}	0.01 ± 0.01	0.46 ± 0.04^{f}	0.01 ± 0.01^{a}
		YC-180	0.01 ± 0.01^{a}	1.28 ± 0.41^{a}	0.08 ± 0.05^{a}	10.05 ± 0.99^{a}	0.04 ± 0.03^{a}	5.69 ± 0.33^{b}	0.24 ± 0.02^{e}	0.01 ± 0.01	0.44 ± 0.06^{f}	0.01 ± 0.01^{a}
		YCX-11	0.01 ± 0.01^{a}	1.40 ± 0.82^{a}	0.09 ± 0.09^{a}	10.29 ± 1.78^{a}	0.05 ± 0.05^{a}	5.91 ± 0.70^{b}	0.25 ± 0.01^{e}	0.01 ± 0.01	0.46 ± 0.03^{f}	0.01 ± 0.01^{a}
	Milk Fat + LA	ABT-5	0.01 ± 0.01^{a}	3.57 ± 0.60^{b}	0.29 ± 0.07^{a}	9.29 ± 1.41^{a}	0.28 ± 0.05^{b}	4.11 ± 0.63^{a}	0.10 ± 0.01^{c}	0.01 ± 0.01	0.01 ± 0.02^{abc}	0.12 ± 0.05^{b}
		YC-180	0.01 ± 0.01^{a}	3.53 ± 0.81^{b}	0.30 ± 0.07^{a}	9.10 ± 1.99^{a}	0.30 ± 0.18^{b}	3.82 ± 0.78^{a}	0.10 ± 0.01^{c}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.12 ± 0.09^{b}
		YCX - 11	0.01 ± 0.01^{a}	3.47 ± 0.63^{b}	0.26 ± 0.06^{a}	9.39 ± 1.88^{a}	0.16 ± 0.09^{ab}	4.11 ± 0.85^{a}	0.07 ± 0.05^{b}	0.01 ± 0.01	0.01 ± 0.01^{ab}	0.03 ± 0.03^{a}
Significance level			p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	NS	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly.

Table 3.6: The effect of fat content, lipid source and culture type on the mono-unsaturated fatty acid content (%) of yogurt.

Fat Content	Lipid source	Culture Type	C14:1c9	C16:1c9	C17:1c10	C18:1t9	C18:1c9	C20:1c11	C24:1
Full Cream Yogurt	Milk Fat (control)	ABT-5	1.03 ± 0.03^{c}	1.20 ± 0.02^{c}	0.09 ± 0.01^{a}	0.19 ± 0.01^{ab}	18.91 ± 0.20^{bcd}	0.47 ± 0.01^{e}	0.01 ± 0.01^{a}
		YC-180	1.05 ± 0.01^{c}	1.20 ± 0.01^{bc}	0.09 ± 0.01^{a}	0.19 ± 0.01^{ab}	18.63 ± 0.15^{bcd}	0.30 ± 0.23^{bcde}	0.01 ± 0.01^{a}
		YCX - 11	1.06 ± 0.02^{c}	0.95 ± 0.37^{b}	0.10 ± 0.02^{a}	0.19 ± 0.01^{ab}	18.57 ± 0.43^{bcd}	0.45 ± 0.01^{de}	0.01 ± 0.01^{a}
	Milk Fat + SFO	ABT-5	0.94 ± 0.03^{c}	1.09 ± 0.02^{bc}	0.08 ± 0.01^{a}	0.17 ± 0.01^{ab}	19.22 ± 0.48^{d}	0.37 ± 0.18^{bcde}	0.01 ± 0.01^{a}
		YC-180	0.98 ± 0.02^{c}	1.13 ± 0.02^{bc}	0.09 ± 0.01^{a}	0.18 ± 0.01^{ab}	19.18 ± 0.20^{d}	0.37 ± 0.18^{bcde}	0.01 ± 0.01^{a}
		YCX - 11	0.99 ± 0.03^{c}	1.14 ± 0.02^{bc}	0.09 ± 0.01^{a}	0.18 ± 0.01^{ab}	19.03 ± 0.45^{cd}	0.30 ± 0.23^{bcde}	0.01 ± 0.01^{a}
	Milk Fat + LA	ABT-5	1.00 ± 0.03^{c}	1.14 ± 0.02^{bc}	0.08 ± 0.01^{a}	0.18 ± 0.01^{ab}	17.95 ± 0.33^{b}	0.22 ± 0.25^{abcde}	0.02 ± 0.03^{a}
		YC-180	1.00 ± 0.01^{c}	1.03 ± 0.28^{bc}	0.08 ± 0.02^{a}	0.18 ± 0.01^{ab}	18.12 ± 0.51^{bc}	0.43 ± 0.04^{cde}	0.04 ± 0.07^{a}
		YCX - 11	1.02 ± 0.02^{c}	1.15 ± 0.01^{bc}	0.10 ± 0.02^{a}	0.18 ± 0.01^{ab}	17.88 ± 0.25^{b}	0.37 ± 0.18^{bcde}	0.01 ± 0.01^{a}
Fat Free Yogurt	Milk Fat (control)	ABT-5	0.56 ± 0.28^{b}	0.99 ± 0.04^{bc}	0.20 ± 0.22^{ab}	1.35 ± 1.05^{c}	21.89 ± 0.87^{f}	0.13 ± 0.13^{ab}	0.01 ± 0.01^{a}
		YC-180	0.52 ± 0.28^{b}	0.98 ± 0.04^{bc}	0.17 ± 0.19^{ab}	0.63 ± 0.98^{abc}	20.54 ± 0.46^{e}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YCX - 11	0.61 ± 0.14^{b}	1.01 ± 0.07^{bc}	0.31 ± 0.16^{bc}	0.96 ± 0.96^{bc}	22.25 ± 0.65^{f}	0.29 ± 0.20^{bcde}	0.01 ± 0.01^{a}
	Milk Fat + SFO	ABT-5	0.09 ± 0.03^{a}	0.19 ± 0.03^{a}	0.25 ± 0.12^{abc}	0.13 ± 0.15^{ab}	23.84 ± 0.15^{g}	0.21 ± 0.01^{abcde}	0.01 ± 0.01^{a}
		YC-180	0.04 ± 0.03^{a}	0.14 ± 0.03^{a}	0.23 ± 0.09^{abc}	0.01 ± 0.01^{a}	23.79 ± 0.25^{g}	0.17 ± 0.03^{abcd}	0.01 ± 0.01^{a}
		YCX-11	0.03 ± 0.06^{a}	0.14 ± 0.09^{a}	0.15 ± 0.08^{ab}	0.01 ± 0.01^{a}	24.15 ± 0.45^{g}	0.17 ± 0.08^{abcd}	0.01 ± 0.01^{a}
	Milk Fat + LA	ABT-5	0.19 ± 0.04^{a}	0.33 ± 0.04^{a}	0.42 ± 0.14^{cd}	0.29 ± 0.32^{ab}	7.06 ± 0.98^{a}	0.15 ± 0.03^{abc}	1.65 ± 0.22^{c}
		YC-180	0.19 ± 0.05^{a}	0.32 ± 0.07^{a}	0.55 ± 0.08^{d}	0.44 ± 0.25^{ab}	6.79 ± 0.76^{a}	0.11 ± 0.06^{ab}	1.69 ± 0.73^{c}
		YCX - 11	0.18 ± 0.04^{a}	0.30 ± 0.06^{a}	0.33 ± 0.06^{bc}	0.28 ± 0.31^{ab}	6.85 ± 0.53^{a}	0.14 ± 0.01^{ab}	1.12 ± 0.21^{b}
Significance level			p <0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly

Table 3.7: The effect of fat content, lipid source and culture type on the poly-unsaturated fatty acid content (%) of yogurt.

Fat Content	Lipid source	Culture Type	C18:2t9,12 (n-6)	C18:2c9,12 (n-6)	C18:3c9,12,15 (n-3)	C18:2c9,t11 (n-6)	C18:2t10,c12 (n-6)	C20:3c11,14,17 (n-3)	C20:3c8,11,14 (n-6)	C20:4c5,8,11,14 (n-6)	C20:5c5,8,11,14,17 (n-3)
Full Cream Yogurt	Milk Fat (control)	ABT-5	0.11 ± 0.06^{b}	1.06 ± 0.49^{a}	0.01 ± 0.01	0.44 ± 0.02^{e}	0.15 ± 0.01^{c}	0.04 ± 0.01^{c}	0.01 ± 0.02	0.05 ± 0.03^{b}	0.01 ± 0.01^{a}
		YC-180	0.13 ± 0.01^{b}	1.22 ± 0.02^{a}	0.15 ± 0.24	0.43 ± 0.01^{e}	0.15 ± 0.01^{c}	0.04 ± 0.01^{c}	0.01 ± 0.01	0.06 ± 0.01^{b}	0.01 ± 0.01^{a}
		YCX -11	0.14 ± 0.01^{b}	1.24 ± 0.08^{a}	0.01 ± 0.01	0.42 ± 0.01^{e}	0.15 ± 0.01^{c}	0.04 ± 0.01^{c}	0.03 ± 0.03	0.03 ± 0.03^{ab}	0.01 ± 0.01^{a}
	Milk Fat + SFO	ABT-5	0.12 ± 0.01^{b}	6.75 ± 0.77^{b}	0.06 ± 0.15	0.39 ± 0.02^{e}	0.13 ± 0.01^{bc}	0.03 ± 0.01^{b}	0.02 ± 0.03	0.03 ± 0.03^{b}	0.02 ± 0.02^{a}
		YC-180	0.13 ± 0.02^{b}	4.89 ± 1.11^{ab}	0.07 ± 0.18	0.41 ± 0.01^{e}	0.14 ± 0.01^{bc}	0.03 ± 0.01^{bc}	0.01 ± 0.02	0.05 ± 0.02^{b}	0.01 ± 0.01^{a}
		YCX -11	0.13 ± 0.01^{b}	4.10 ± 1.17^{ab}	0.15 ± 0.23	0.41 ± 0.01^{e}	0.14 ± 0.01^{bc}	0.04 ± 0.01^{bc}	0.01 ± 0.02	0.05 ± 0.02^{b}	0.01 ± 0.01^{a}
	Milk Fat + LA	ABT-5	0.13 ± 0.01^{b}	5.57 ± 1.08^{ab}	0.21 ± 0.23	0.40 ± 0.02^{e}	0.15 ± 0.01^{c}	0.04 ± 0.01^{bc}	0.01 ± 0.01	0.06 ± 0.01^{b}	0.01 ± 0.01^{a}
		YC-180	0.13 ± 0.01^{b}	5.11 ± 0.34^{ab}	0.01 ± 0.01	0.42 ± 0.02^{e}	0.15 ± 0.01^{c}	0.04 ± 0.01^{c}	0.01 ± 0.02	0.05 ± 0.02^{b}	0.01 ± 0.01^{a}
		YCX -11	0.13 ± 0.01^{b}	4.81 ± 0.89^{ab}	0.07 ± 0.18	0.42 ± 0.01^{e}	0.14 ± 0.01^{bc}	0.04 ± 0.01^{bc}	0.01 ± 0.01	0.06 ± 0.01^{b}	0.01 ± 0.01^{a}
Fat Free Yogurt	Milk Fat (control)	ABT-5	0.01 ± 0.01^{a}	2.42 ± 0.44^{ab}	0.01 ± 0.01	0.24 ± 0.13^{d}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YC-180	0.01 ± 0.01^{a}	2.35 ± 0.33^{ab}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YCX-11	0.01 ± 0.01^{a}	3.12 ± 1.31^{ab}	0.01 ± 0.01	0.17 ± 0.14^{cd}	0.10 ± 0.11^{bc}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.07 ± 0.18^{abc}
	Milk Fat + SFO	ABT-5	0.01 ± 0.01^{a}	54.01 ± 2.03^{c}	0.01 ± 0.01	0.04 ± 0.02^{ab}	0.01 ± 0.02^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.11 ± 0.02^{abc}
		YC-180	0.01 ± 0.01^{a}	57.08 ± 1.79^{c}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.08 ± 0.05^{abc}
		YCX-11	0.01 ± 0.01^{a}	56.23 ± 3.85^{c}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.07 ± 0.06^{abc}
	Milk Fat + LA	ABT-5	0.01 ± 0.01^{a}	69.12 ± 4.17^{d}	0.01 ± 0.01	0.13 ± 0.03^{bc}	0.11 ± 0.01^{bc}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.13 ± 0.06^{bc}
		YC-180	0.01 ± 0.01^{a}	69.41 ± 5.71^{d}	0.02 ± 0.05	0.12 ± 0.02^{bc}	0.10 ± 0.01^{bc}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.18 ± 0.11^{c}
		YCX -11	0.01 ± 0.01^{a}	70.74 ± 4.24^{d}	0.01 ± 0.01	0.08 ± 0.06^{abc}	0.08 ± 0.06^{b}	0.01 ± 0.01^{a}	0.01 ± 0.01	0.01 ± 0.01^{a}	0.04 ± 0.04^{ab}
Significance level			p < 0.001	p < 0.001	NS	p < 0.001	p < 0.001	p < 0.001	NS	p < 0.001	p < 0.001

NS = Not Significant.

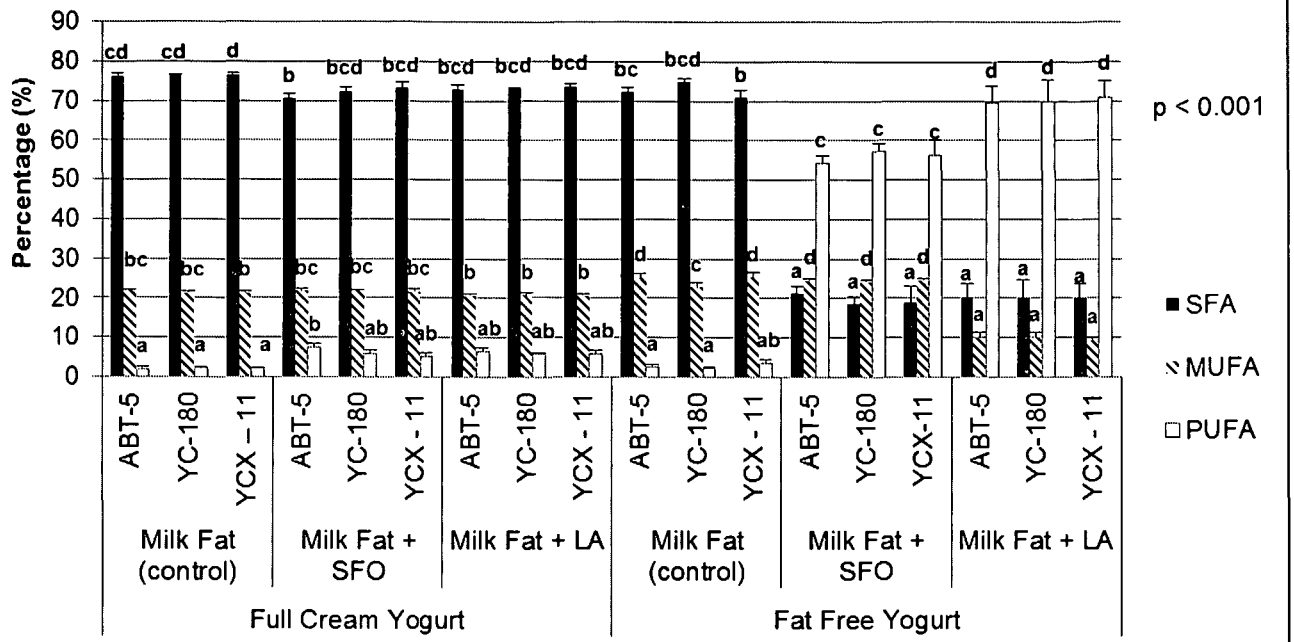
Means with different superscripts in the same column differ significantly.

No significant differences occurred in the concentrations of the CLA1 and CLA2 isomers of the full cream yogurt samples from different lipid sources. This might have been due to the CLA already present in milk fat. The fat free yogurt control samples had significantly ($p < 0.001$) higher levels of the CLA1 isomer, but lower concentrations of the CLA2 isomer. Compared to the other lipid sources milk fat usually contains higher levels of CLA1 than CLA2 (Peterson *et al.*, 2002). It is possible that a difference in the CLA1 could have occurred between the fat free yogurt samples, but it was not detected due to the already present CLA1 isomer in milk fat (Peterson *et al.*, 2002).

Fatty acid ratios

The SFA content of all the full cream yogurt samples and the fat free yogurt control sample were essentially the same, but differed significantly ($p < 0.001$) from the fat free yogurt samples that were supplemented with LA and SFO (Fig. 3.3). The exception was the fat free control yogurt manufactured from the YC-X11 starter culture that had a statistically significant ($p < 0.001$) lower SFA content than the full cream control yogurt manufactured from starter cultures ABT-5, YC-180 and YC-X11. Bovine milk is relatively high in SFA's, but the total fat content of the fat free yogurt was very low and the FA ratios of the fat free yogurt were therefore more easily affected by the addition of the high levels of PUFA's contained in the LA and SFO that were used (Belitz & Grosch, 2008). The fat free yogurt samples that were supplemented with LA and SFO had a statistically significant ($p < 0.001$) lower ratio of SFA's and a higher ratio of PUFA's than the fat free control and full cream yogurt samples (Fig. 3.3).

The fat free yogurt supplemented with LA had a significantly ($p < 0.001$) lower MUFA ratio compared to the rest of the yogurt samples. Bovine milk fat contains MUFA's and the SFO that was used also contains MUFA's, but the LA product that was used contained an insignificant amount of MUFA's. That explains the lower MUFA ratio that was obtained for the fat free yogurt supplemented with LA compared to the other fat free yogurt samples and full cream yogurt samples (Belitz & Grosch, 2008).



(a,b) Means with different superscripts differ significantly

Figure 3.3 Influence of the different treatments on the fatty acid ratios of the yogurt.

Actual conjugated linoleic acid (CLA) content

Table 3.8 indicates that the fat from the full cream yogurt samples had significantly ($p < 0.001$) higher levels of both the CLA isomers (CLA1 and CLA2) compared to the fat of the fat free yogurt samples. Conjugated linoleic acid forms part of the total lipid of the product and due the higher total fat content of the full cream yogurt, the CLA concentration of both the CLA1 and CLA2, was higher than the CLA isomer concentration of the fat free yogurt (Lin *et al.,* 1995; Lin *et al.,* 1998; Lin *et al.,* 1999; Prandini *et al.*, 2007).

Significant ($p < 0.001$) differences in the CLA1 and CLA2 isomer concentrations between the fat from the control yogurt samples, the fat from the yogurt with LA and the yogurt with SFO were obtained (Table 3.8). The highest CLA1 concentration in the full cream yogurt was obtained with the addition of SFO and in the fat free yogurt the highest CLA1 concentration was obtained with the addition of LA (Table 3.8). Differences was however very small. The highest CLA2 concentration in both the fat free yogurt and the full cream yogurt was obtained with LA supplementation.

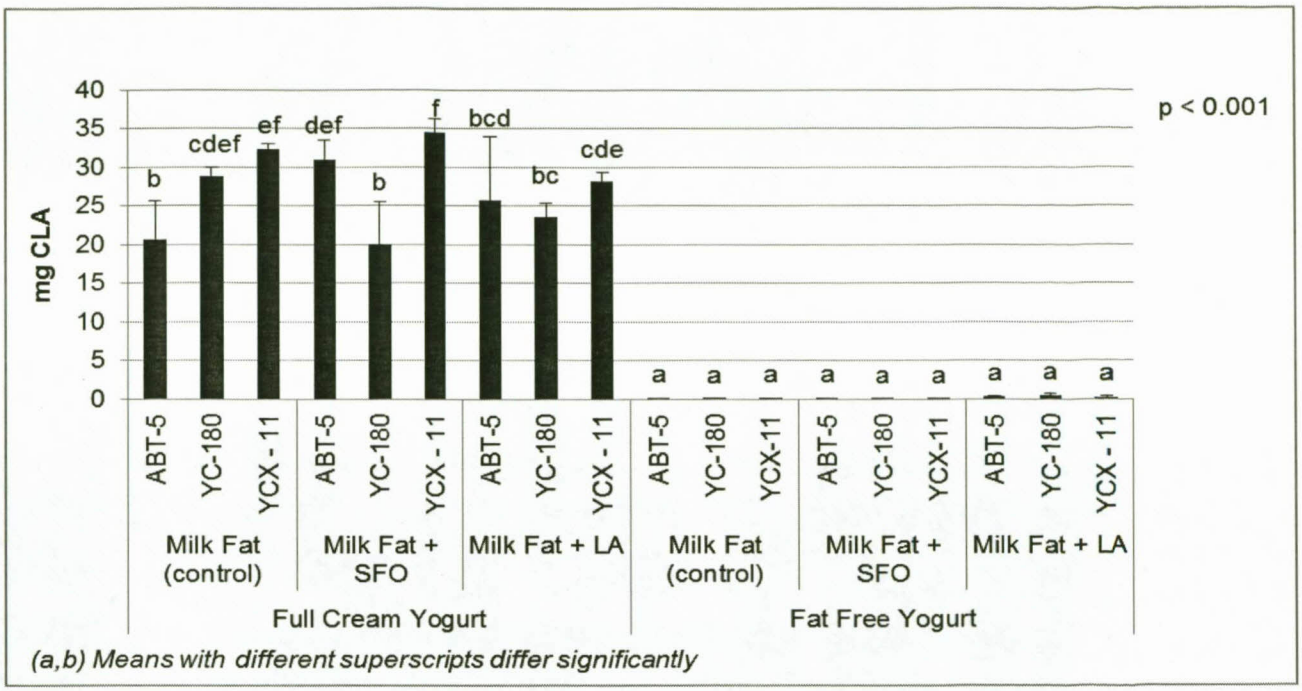
Table 3.8: The effect of fat content, lipid source and culture type on the actual conjugated linoleic acid content of yogurt.

Fat Content	Lipid source	Culture Type	mg CLA1 (C18:2c9t11)/g fat	mg CLA2 (C18:2t10c12)/g fat
Full cream Yogurt	Milk Fat (control)	ABT-5	5.31 ± 1.16^{cd}	1.88 ± 0.47^{fg}
		YC-180	7.40 ± 0.24^{efg}	2.54 ± 0.09^{hij}
		YCX-11	8.58 ± 0.16^{g}	3.01 ± 0.09^{j}
	Milk Fat + SFO	ABT-5	7.24 ± 0.25^{efg}	2.52 ± 0.10^{hij}
		YC-180	4.68 ± 1.27^{c}	1.53 ± 0.38^{ef}
		YCX-11	8.41 ± 0.23^{fg}	2.88 ± 0.16^{ij}
	Milk Fat + LA	ABT-5	6.08 ± 1.89^{de}	2.34 ± 0.81^{ghi}
		YC-180	5.64 ± 0.43^{cd}	2.05 ± 0.24^{fgh}
		YCX-11	7.11 ± 0.08^{ef}	2.41 ± 0.06^{ghij}
Fat free Yogurt	Milk Fat (control)	ABT-5	0.52 ± 0.27^{ab}	0.01 ± 0.01^{a}
		YC-180	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YCX-11	0.41 ± 0.35^{ab}	0.26 ± 0.28^{abc}
	Milk Fat + SFO	ABT-5	0.38 ± 0.20^{ab}	0.09 ± 0.19^{ab}
		YC-180	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		YCX-11	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
	Milk Fat + LA	ABT-5	0.94 ± 0.29^{ab}	0.79 ± 0.23^{cd}
		YC-180	1.44 ± 0.55^{b}	1.14 ± 0.29^{de}
		YCX-11	0.65 ± 0.50^{ab}	0.66 ± 0.54^{bcd}
Significance level			$p < 0.001$	$p < 0.001$

(a,b) Means with different superscripts in the same column differ significantly

Significant ($p < 0.001$) differences in the concentration of both CLA isomers (CLA1 and CLA2) were obtained between the yogurt manufactured from different starter cultures (Table 3.8). In the case of the full cream yogurt control, the full cream yogurt supplemented with LA and full cream yogurt supplemented with SFO, the highest CLA concentration of both CLA isomers (CLA1 and CLA2) was obtained with the use of the YC-X11 starter culture. With the fat free yogurt the situation was less clear. The highest CLA concentration of both the CLA isomers (CLA1 and CLA2) in the fat free yogurt supplemented with LA was obtained with the use of the YC-180 starter culture. The activities of the desaturase enzymes in the different starter cultures differed and might also have been influenced by the FA's contained in the milk fat, LA and the SFO, resulting in differences in the concentrations of the CLA1 and CLA2 isomers obtained with the use of the different starter cultures (Sieber *et al.*, 2004).

In Fig. 3.4 it is shown that the full cream yogurt samples had significantly ($p < 0.001$) higher levels of total CLA per 100 g of yogurt. Lipid content was therefore an important factor influencing the final CLA level in the yogurt products. CLA forms part of the total lipid in the yogurt; therefore it was also not surprising to find a positive relationship between the



(a,b) Means with different superscripts differ significantly

Figure 3.4 Effect of the different treatments on the total CLA concentration in 100 g of yogurt.

CLA content and the total lipid content (Lin *et al.,* 1995, 1998, 1999; Prandini *et al.*, 2007). Full cream yogurt naturally contains higher protein levels than fat free yogurt and the protein neutralized the inhibitory effect on the LAB by the FA's (Boyaval *et al.*, 1995; Lin *et al.*, 1999). The LAB in the fat free yogurt were less protected and the bioconversion process of LA to CLA may be inhibited to a certain degree in the fat free yogurt (Lin *et al.*, 1999).

The fat free yogurt with added LA had a slightly higher total CLA concentration per 100 g of yogurt compared to the fat free yogurt control and the fat free yogurt that was supplemented with SFO (Fig. 3.4). The amount of PUFA's present was one important component which had a strong correlation with the presence CLA in the milk product (Peterson *et* al., 2002). SFO, which contained high levels of PUFA's might have caused the LAB in the starter cultures to produce CLA. The SFO acted as a slow-release source of LA, which was then converted to CLA (Peterson *et al.*, 2002).

In a study by Lin *et al.* (1999), *L. acidophilus, L. delbrueckii* spp. *bulgaricus, L. delbrueckii* spp. *lactis, L. lactis,* spp. *cremoris, L. lactis,* spp. *lactis and S. salivarius* spp. *thermophilus,*

all demonstrated the ability to convert LA to CLA. The LAB enzymes were capable of converting LA to CLA due to the possible presence of desaturase activity (Lin, 2006).

Significant ($p < 0.001$) differences in the total CLA contained in 100 g yogurt were observed between the yogurt samples manufactured with the different yogurt starter cultures (Fig. 3.4). In the case of the full cream yogurt control, the full cream yogurt supplemented with LA and full cream yogurt supplemented with SFO, the highest total CLA concentration was obtained with the use of the YC-X11 starter culture. The highest total CLA concentration in the fat free yogurt was obtained with the use of the YC-180 starter culture. The level of long chain FA's must be kept low, as a too high level can be inhibitory not only to cell growth of bacteria, but also to the biohydrogenation activity of the bacterial enzymes (Kim & Liu, 2002). This might also have caused the different total CLA concentrations obtained with the use of the different starter cultures. The starter cultures that were used might also not have been equally protected from the inhibitory effects of the LA (Kim & Liu, 2002; Sieber *et al.*, 2004).

The pH is lowered by the formation of lactic acid and the CLA production in the LAB cells could have been stopped as the lower pH could have been an additional cause for the inactivation of the isomerase activity (Kim & Liu, 2002). This might explain the difference in CLA levels between the yogurts manufactured with different starter cultures. Some starter cultures produced more lactic acid in a shorter time than others and therefore also produced less CLA. It is possible that more CLA could have been produced with a longer fermentation time. According to studies by Lin (2006) it appeared that the LA isomerase activity of *L. delbrueckii* spp. *bulgaricus* was lower than the isomerase activity of *L. acidophilus.* The lactic acid formation during fermentation caused the pH to drop below 4.6, causing curd formation and it interfered with free access of the enzyme to the bacterial substrate.

3.4 CONCLUSIONS

This experiment was novel in the sense that commercial mixtures of starter cultures were used to determine the effect on CLA fortification in yogurt. In previous studies selected LAB strains under controlled conditions in laboratory media were used. Therefore results from this study cannot be completely compared to results from previous studies.

The results from this experiment proved that neither the total fat content of the full cream yogurt nor the total fat content of the fat free yogurt was significantly influenced by the addition of LA or SFO. This means that even with the supplementation of LA and SFO to increase the CLA content of the yogurt, the initial total fat content of the milk from which the yogurt is made, can still be maintained.

The LA and SFO that was added to the milk for the yogurt manufacture did influence the FA profile of the final yogurt product significantly. There was an overall decrease in the SFA's and MUFA's and an increase in the total PUFA's. This influence of the LA sources on the FA profile on the yogurt may be regarded as positive, as in most cases the SFA's are negatively associated with its effects on human health.

Significant differences were detected in the total CLA content of the yogurt that was fermented with the three different starter cultures. This was an indication that certain starter cultures have the ability to convert more LA to CLA. This may be due to the fact that some LAB contained in the different starter cultures may have been more sensitive to the lowered pH and the antimicrobial effects of LA, which inhibited the activities of the isomerase enzyme which is responsible for the conversion of LA to CLA. Overall the total fat content of the yogurt, the LA source that was added and the type of starter culture used for the yogurt manufacturing process and the interaction between them, had a significant effect on the total CLA content of the final yogurt product. The highest CLA value was obtained in the full cream yogurt and it was with the addition of SFO and the use of YCX-11 which contains *S. salivarius* spp. *thermophilus* and *L. delbrueckii* spp. *bulgaricus.* These strains have also been identified by authors in previous studies (Jiang *et al.*, 1998; Lin *et al.*, 1999; Pariza & Yang, 1999; Lin, 2000; Ogawa *et al.*, 2001; Ham *et al.,* 2002; Kim & Liu, 2002; Alonso *et al.,* 2003; Coakley *et al.,* 2003; Kishino *et al.,* 2003; Lin, 2003; Lin *et al.*, 2003; Lin, 2006) to be among the LAB with the highest potential in converting LA to CLA. The necessity for LA as an additive is a great disadvantage for the starter culture approach.

Linoleic acid and CLA may have a negative influence on the flavour of the fermented milk product and the characteristic flavour is an important factor that can influence the quality attributes of the yogurt. The use of a pure LA product may also increase the yogurt product costs significantly. Since high levels of CLA could be obtained with the addition of SFO

rather than LA, it is possible to manufacture yogurt with naturally increased CLA levels without an enormous change in product costs.

Although the approach to naturally increase the CLA levels was successful, the amount of CLA obtained in this yogurt with elevated CLA levels is still not sufficient to supply maximum health benefits. An amount of approximately 15 kg of this yogurt with increased CLA produced by or from starter cultures, needs to be consumed per day to reach the total recommended dietary allowance (RDA) for CLA.

In order to create a yogurt product with CLA levels high enough to meet the RDA by consuming one portion to three portions of yogurt, other methods of fortification must rather be considered.

CHAPTER 4

THE EFFECTS OF SYNTHETIC CONJUGATED LINOLEIC ACID FORTIFICATION ON YOGURT SENSORY PROPERTIES, STABILITY AND SHELF-LIFE

ABSTRACT

The aim of this study was to determine the effect of fortifying yogurt with synthetic conjugated linoleic acid (Tonalin® 60-WDP), on yogurt quality, sensory properties, stability and shelf-life of low fat strawberry flavoured yogurt. Conjugated linoleic acid fortified yogurt was prepared by supplementing yogurt with appropriate amounts of Tonalin® 60-WDP to create yogurt with 0 %, 25 %, 50 % and 100 % of the recommended dietary allowance for conjugated linoleic acid respectively. The Tonalin® 60-WDP inclusion significantly ($p < 0.001$) increased the total fat content of the yogurt. The fatty acid composition of the yogurt was significantly ($p < 0.001$) influenced by the Tonalin® 60-WDP inclusion levels. With increased Tonalin® 60-WDP, the polyunsaturated fatty acid content increased and saturated fatty acids and mono-unsaturated fatty acids decreased significantly. The concentration of the C18:2c9,t11 and C18:2t10,c12 conjugated linoleic acid isomers were significantly ($p < 0.001$) increased in the yogurt with increased Tonalin® inclusion levels ($p < 0.001$). Tonalin® inclusion had no significant effect on the viability of the yogurt lactic acid bacteria. The yogurt with the Tonalin® of all four treatment levels were accepted by the consumer panel. With the addition of Tonalin® a more stable yogurt product with increased health benefits was created.

Keywords: conjugated linoleic acid, sensory; synthetic; Tonalin® 60-WDP

4.1 INTRODUCTION

Since the beginning of mankind people consumed food to satisfy their hunger and energy needs. Over the years the importance of the type of food product and its nutritional properties became more important. Food is not intended to only satisfy hunger and to

provide necessary nutrients anymore, but also to prevent nutrition-related diseases and to improve physical and mental well-being (Roberfroid, 2000b; Menrad, 2003). This tendency has led to the need for more improved food products as consumers believe more and more that food contribute directly to their health (Young, 2000; Mollet & Rowland, 2002).

For years yogurt has been seen as a food product with major health benefits. In the studies by a Russian bacteriologist, Eli Metchnikoff, he discovered that the people in Bulgaria, who consumed a lot of yogurt, tended to live longer than people from other countries with lower yogurt consumption (Hughes & Hoover, 1991).

The increased demand for functional foods can be explained by the increasing costs of healthcare, the steady increase in life expectancy and the desire for older people for improved quality of life during their later years (Roberfroid, 2000a; Roberfroid, 2000b; Kotilainen *et al.*, 2006). The term "functional food" originated in Japan in the 1980's for food products fortified with special constituents that possess advantageous physiological effects (Hardy, 2000; Kwak & Jukes, 2001; Stanton *et al.*, 2005). The dairy-based beverages market may be seen as the market of main focus when the sales of yogurt, milk and other beverages are taken into consideration. Dairy beverages containing probiotics and or prebiotics dominate the functional dairy beverage market, but the focus recently also moved to conjugated linoleic acid (CLA) enriched dairy products (Gagada *et al.*, 1999).

Conjugated linoleic acid is a group of polyunsaturated fatty acids (PUFA's), existing as a mixture of positional and geometric isomers of octadecadienoic acid [linoleic acid (LA), 18:2n-6] (Chin *et al.*, 1992; Lin *et al.*, 1995; Parodi, 1997) which can have a major positive contribution to human health. Studies have shown that CLA has the ability to inhibit growth in a number of human cancer cell lines. Conjugated linoleic acid is also anti-atherogenic, immune-modulating and growth promoting (Bhattacharye *et al.*, 2006). Conjugated linoleic aicd was found to decrease fat mass without significantly affecting body weight (Bhattacharye *et al.*, 2006; Akalin *et al.*, 2007). Not only did CLA depress total cholesterol, but it also lowered the low density lipoprotein (LDL) to high density lipoprotein (HDL) cholesterol ratio significantly in a study by Lin *et al.* (1999). The positive health contribution CLA can have is the main reason why dairy consumption became a strong recommendation as part of the human diet since bovine milk was found to be the richest natural source of CLA (Bhattacharye *et al.*, 2006; Akalin *et al.*, 2007).

The average level of CLA in the fat of fresh full cream bovine milk is approximately 4.45 mg/g fat (Campbell *et al.*, 2003; Khanal & Olson, 2004). The average amount of CLA contained in the fat of bovine dairy products in general is in the range of 0.55 to 9.12 mg/g fat, depending on the specific product (Akalin *et al.*, 2007). The amount of CLA contained in bovine dairy products, is still too low to have maximum health benefits and approximately 40 liters of fresh full cream milk must be consumed per day to reach the full recommended dietary allowance (RDA) (Akalin *et al.*, 2007).

It is estimated that a 70 kg human should consume about 3.0 to 3.5 g of CLA per day in order to reach maximum health benefits (Rodríguez-Alcalá & Fontecha, 2007; Akalin *et al.*, 2007; Hur *et al.*, 2007). This is more than three times the daily consumption of the average adult according to Rodríguez-Alcalá & Fontecha (2007). Therefore it became necessary to increase the CLA levels in food products (Hur *et al.*, 2007).

Linoleic acid can be added to milk to be converted to CLA by certain strains of lactic acid bacteria (LAB) used as starter cultures during the manufacturing process of fermented milk products (Kim & Liu, 2002). In the previous chapter the possibility to elevate yogurt CLA levels through the conversion of LA to CLA by LAB in yogurt starter cultures was studied. Increased CLA concentrations were obtained in the study, but far below the RDA. Approximately 15 kg (150 portions) of the yogurt from this study, with elevated CLA concentrations must be consumed per day to reach the RDA. Other methods of fortification must rather be considered to meet the RDA for CLA.

Conjugated linoleic acid levels in dairy products can be elevated through direct supplementation of synthetic CLA (e.g. Tonalin® 60-WDP) (Tamime & Robinson, 2001; Rodríguez-Alcalá & Fontecha, 2007; Jimenez *et al.*, 2008).

The aim of this experiment was to determine the effect of Tonalin®, (a synthetic microencapsulated CLA product) fortification of yogurt on its sensory properties, stability and shelf-life.

4.2 MATERIALS AND METHODS

SAMPLE PREPARATION

Strawberry flavoured yogurt was used, because it is currently the most popular yogurt flavour in South Africa (Slabber, 2011, personal communication). Base low fat yogurt and strawberry syrup concentrate was provided by Dairybelle (Bloemfontein, South Africa). The CLA yogurt was prepared on site at Dairybelle. Strawberry syrup was added to the base yogurt in a final concentration of 12 % and thoroughly mixed with a Kenwood electrical mixer.

The Tonalin® 60-WDP (water soluble powder) from Cognis was added in amounts of 0 g (0 %), 25 g (1.25 %), 50 g (2.5 %) and 100 g (5 %) Tonalin® to 2 kg yogurt to create the CLA fortified yogurt products, representing 0 % (control), 25 %, 50 % and 100% of the RDA for CLA, respectively. After the Tonalin® was added, the yogurt was thoroughly mixed with the Kenwood electrical mixer for about 2 to 3 minutes until all the powder was dissolved in the yogurt. The yogurt was divided, packed and sealed by Dairybelle in 100 g yogurt tubs. The 100 g yogurt samples with the different Tonalin® treatment levels were stored at 4°C and analyzed after three storage time intervals: day 0, week 3 and week 6. Analysis on six 100 g replicates of each of the Tonalin® treatment levels were done for all three storage time intervals.

MICROBIAL AND CHEMICAL ANALYSIS

Total lactic acid bacterial (LAB) counts

For the determination of LAB, 10 g from each 100 g yogurt sample was weighed off in a stomacher bag (Whirl Pak TM), 90 ml 0f 0.1 M phosphate buffer was added and the sample was homogenized (Lab Blender 400, ART Medical Equipment) for 1 min. Further serial dilutions were prepared to 10^{-10} and 1 ml of the appropriate dilutions was plated by the pour-plate method and using MRS agar (Oxoid CM 0361; de Man et al., 1960). Plates were incubated at 32 °C for 72 h. After incubation the colonies were enumerated by means of a colony counter and the interpretation of the counts was done using standard microbial techniques (Harrigan, 1998).

Water activity (A_w)

The water activity (A_w) was measured with a Novasina TH-2 A_w meter. The A_w for the yogurt with all four the Tonalin® treatment levels of the three storage time intervals were determined.

pH

The pH was measured using an Orion pH-302 bench top pH meter. The pH meter was first calibrated with Hanna pH 4 solution followed by the pH 7 solution. The pH was determined on yogurt samples from all four Tonalin® treatment levels and three storage time intervals.

Viscosity

The viscosity was measured with a Brookfield DV-11 viscometer by using a size "4" spindle for more viscous fluids. The viscosity was measured for all the yogurt samples in Centipoise (CPS) (mPa/sec).

Oxidative stability

Oxidative stability as measured by mg malonaldehyde/kg yogurt was assessed by determining thiobarbituric acid reactive substances (TBARS) using the aqueous acid extraction method of Raharjo *et al.* (1992).

PROXIMATE ANALYSIS

Fat extraction

Total lipid from the 72 yogurt samples were quantitatively extracted, according to the method of Folch *et al.* (1957) using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene was added at a concentration of 0.001 % to the chloroform: methanol mixture. A rotary evaporator was used to dry the fat extracts under

vacuum and the extracts were also dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as moisture adsorbent.

Total extractable fat content (EFC) was determined gravimetrically and expressed as % fat (w/w) per 100 g yogurt. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction, after drying. By determining the difference in weight, the FFDM could be expressed as % total solids (TS) (w/w) per 100 g yogurt. The moisture content of the yogurt was determined by subtraction (100 % - % lipid - % TS) and expressed as % moisture (w/w) per 100 g of yogurt. The extracted fat was stored in a polytop (glass vial, with push-in top) under a blanket of nitrogen and frozen at –20°C until further analyzed.

Fatty acid analysis

The lipid (from Folch extraction) was transferred into a Teflon-lined screw-top test tube by means of a disposable glass pasteur pipette. Fatty acids (FA's) were transesterified to form methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride in methanol (Park & Goins, 1994). CLA isomers were quantitatively determined by using heptadecanoic acid (C17:0) as internal standard. C18:2c9t11 (CLA1) and C18:2t10c12 (CLA2) isomers could then be expressed as mg CLA isomer/g fat. The areas of the CLA isomers were expressed against the area of the internal standard. Correction factors for different CLA isomers were also calculated. Peak identification of the FA's and the FA profile were done with an external standard, Supelco 37 component FAME Mix.

Fatty acid methyl esters (FAME) were quantified using a Varian GX 3400 flame ionization Gas Chromatograph (GC), with a fused silica capillary column (Chrompack CPSIL 88 - 100 m length, 0.25 mm ID, 0.2 μm film thickness). Column temperature was 40 to 230°C (hold 2 min; 4°C/min; hold 10 min).

Fatty acid methyl esters in hexane (1 μl) were injected into the column using a Varian 8200 CX Autosampler with a split ratio of 100:1. The injection port and detector were both maintained at 250°C. Hydrogen, at 45 psi, functioned as the carrier gas, while nitrogen was employed as the makeup gas. Varian Star Chromatography Software recorded the

chromatograms. FAME samples were identified by comparing the relative retention times of FAME peaks from samples with those of standards obtained from SIGMA (189-19).
Fatty acids were expressed as the relative percentage of each individual FA as a percentage of the total of all FA's present in the sample and the CLA could be expressed as mg CLA isomer/ 100 g of yogurt.

STATISTICAL ANALYSIS

The experimental design is a 4 X 3 factorial design, with four Tonalin® inclusion levels and three storage intervals. An analysis of variance (ANOVA) procedure for balanced data (NCSS, 2007) was used to determine the effect of CLA (Tonalin®) inclusion level, storage time and their interactions on proximate composition, microbial stability, stability, FA composition, FA ratios and actual CLA content of yogurt. A one way analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine whether the above mentioned variables were significantly influenced by the 12 main treatment groups. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) were carried out to determine whether significant differences exist between treatment means (NCSS, 2007).

SENSORY ANALYSIS

A consumer panel of 75 selected regular yoghurt consumers was asked to assemble at the Sensory Facility of the University of the Free State to taste/evaluate and give their acceptability opinion on the four yogurt variants. The term "regular" implicated that they had to eat yoghurt at least three times a week. The panel comprised of 18 females and 57 males, with the ages ranging from < 20 to > 60 years.

For both panels, groups of the five respondents convened every 15 min to taste the four samples. The questionnaire consisted of a basic nine-point hedonic scale (Fig. 4.1) and was amended to include the attributes of overall liking, taste, mouthfeel and aftertaste (Annexure A). Respondents were asked to respond to the question "how much do you like or dislike the sample?". All samples were coded with randomized, 3-digit codes and rotated to prevent bias. Each respondent received a covered miniature plastic container with 15 ml of yogurt sample per variant. Samples were kept at room temperature for 30 min before serving. Bottled water, at room temperature, was used as palate cleanser.

Instruction:

Please indicate with an X how much you like or dislike the product OVERALL.

Sample code: _____

OVERALL LIKING

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Figure 4.1 Nine-point hedonic scale

Evaluations were performed in individual tasting booths and under white lights, as there were no color differences to be masked.

All the data was collected in spread sheets using Microsoft Excel 2007 and all the statistical analyses were done using NCSS (2007). The significance of the overall acceptance measured for each yogurt variant, was tested by means of analysis of variance (ANOVA). The different variants were used as the main effects at a significance level of 95 % ($p \leq 0.05$). If the main effect was significant, the Tukey Kramer Multiple Comparison Test was applied to determine the direction of the differences between mean values.
The panel had to make use of a nine-point hedonic scale (Fig. 4.1) which included the attributes of taste, mouthfeel, aftertaste and overall liking. The scores ranged from 1 for dislike extremely to 9 for like extremely.

4.3 RESULTS AND DISCUSSIONS

The analysis of variance (ANOVA) for the effect of the Tonalin® inclusion level and storage time and the interaction for Tonalin® inclusion level X storage time on the proximate composition, FA composition, FA ratios and the actual CLA content of the fortified yogurt is shown in Table 4.1.

In Table 4.1 it can be seen that the Tonalin® inclusion level was the only factor that significantly ($p < 0.001$) influenced the total fat content of the yogurt. The total moisture content and TS content were significantly ($p < 0.001$) influenced by both the Tonalin®

Table 4.1: Analysis of variance (ANOVA) on proximate composition, microbial and chemical stability, fatty acid composition, fatty acid ratios and actual conjugated linoleic acid content for the effect of CLA inclusion level and storage time and their interactions.

	Inclusion Level	Storage Time	Inclusion Level X Storage Time
Proximate analysis:			
Fat content (%)	***	NS	NS
Moisture content (%)	***	***	NS
Solids content (%)	**	***	NS
Microbial and Chemical Stability:			
log cfu LAB/ ml	NS	***	NS
Water Activity (A_w)	***	NS	NS
pH	***	***	***
Viscosity CPS (mPa/sec)	***	***	***
TBARS value	***	***	*
Fatty Acid Composition (%):			
C4:0	***	NS	NS
C6:0	***	NS	NS
C8:0	***	NS	NS
C10:0	***	NS	NS
C12:0	***	NS	NS
C13:0	***	NS	NS
C14:0	***	NS	NS
C14:1c9	***	NS	NS
C15:0	***	NS	NS
C16:0	***	NS	NS
C16:1c9	***	NS	NS
C17:0	***	NS	NS
C17:1c10	***	NS	NS
C18:0	***	NS	NS
C18:1t9	***	NS	NS
C18:1c9	***	NS	NS
C18:1c7	***	NS	NS
C18:2t9,12 (n-6)	NS	NS	NS
C18:2c9,12 (n-6)	***	NS	NS
C18:3c9,12,15 (n-3)	***	NS	NS
C18:2c9,t11 (n-6) (CLA1)	***	NS	NS
C18:2t10,c12 (n-6) (CLA2)	***	NS	NS
Total CLA	***	NS	NS
C20:0	***	NS	NS
C20:3c11,14,17 (n-3)	***	NS	NS
C20:4c5,8,11,14 (n-6)	***	NS	NS
C21:0	***	NS	NS
C22:0	***	NS	NS
Fatty Acid Ratios (%):			
SFA	***	NS	NS
MUFA	***	NS	NS
PUFA	***	NS	NS
n-3	***	NS	NS
n-6	***	NS	NS
Actual conjugated linoleic acid content:			
C18:2c9,t11 (n-6) (CLA1) (mg/ g fat)	***	NS	NS
C18:2c9,t11 (n-6) (CLA1) (mg/ 100g yogurt)	***	NS	NS
C18:2t10,c12 (n-6) (CLA2) (mg/ g fat)	***	NS	NS
C18:2t10,c12 (n-6) (CLA2) (mg/ 100 g yogurt)	***	NS	NS
Total conjugated linoleic acid (mg/ g fat)	***	NS	NS
mg Total CLA/ 100 g yogurt	***	NS	NS

NS = Not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

inclusion level and the storage time. The interaction of inclusion level and storage time had no significant ($p < 0.001$) influence in the proximate composition of the yogurt.

Storage time was the only parameter that had a significant ($p < 0.001$) influence on the total LAB (log cfu LAB/ ml) counts. The A_w was significantly ($p < 0.001$) influenced by only the Tonalin® inclusion level. The Tonalin® inclusion level, the storage time and the interaction for inclusion level and storage time had a significant ($p < 0.001$) influence on the yogurt pH and viscosity. Oxidative stability (TBARS) of the yogurt was significantly ($p < 0.001$) influenced by both inclusion level and storage time as well as the interaction ($p < 0.05$) between inclusion level and storage time. The Tonalin® inclusion level significantly ($p < 0.001$) influenced the FA composition, FA ratios and actual CLA content. The only exception was C18:2t9,12 that was not influenced significantly ($p < 0.001$) by inclusion level.

None of the FA parameters were significantly ($p < 0.001$) influenced by storage time or by the interaction between inclusion level and storage time.

PROXIMATE COMPOSITION

The total fat content increased significantly ($p < 0.001$) with increased Tonalin® inclusion level (Fig. 4.2). This trend was observed for all three the sampling time intervals since the storage time had no significant ($p < 0.001$) influence on the total fat content. The average total fat content of the control yogurt samples were approximately 2 %, which is the standard average total fat concentration of low fat yogurt (Belitz & Grosch, 2008). The fat content of the yogurt increased to more than 5 % for the yogurt with 5 % Tonalin® inclusion level (Fig. 4.2). The increase in the yogurt total fat content with increased Tonalin® inclusion levels was due to the fact that the Tonalin® product contained a total fat content of approximately 76.5 % (Cognis, 2004).

A slight but not significant increase in the TS content was observed with the increased Tonalin® inclusion levels (Fig. 4.3). This was to be expected as the microcapsule of the encapsulated CLA in the Tonalin®, consisted of skim milk powder and caseinates. The proteins and carbohydrates contained in the skim milk powder, contributed to the increase in the total non-fat solids content of the yogurt supplemented with Tonalin®.

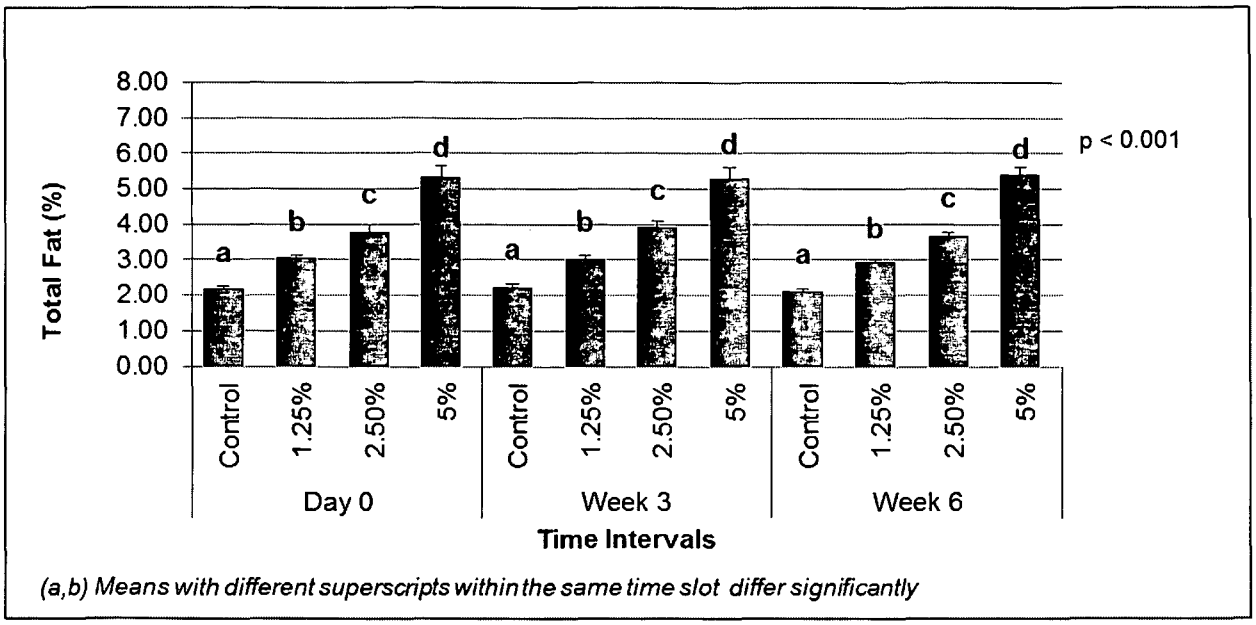


Figure 4.2 Yogurt total fat content as affected by the Tonalin® 60-WDP inclusion level.

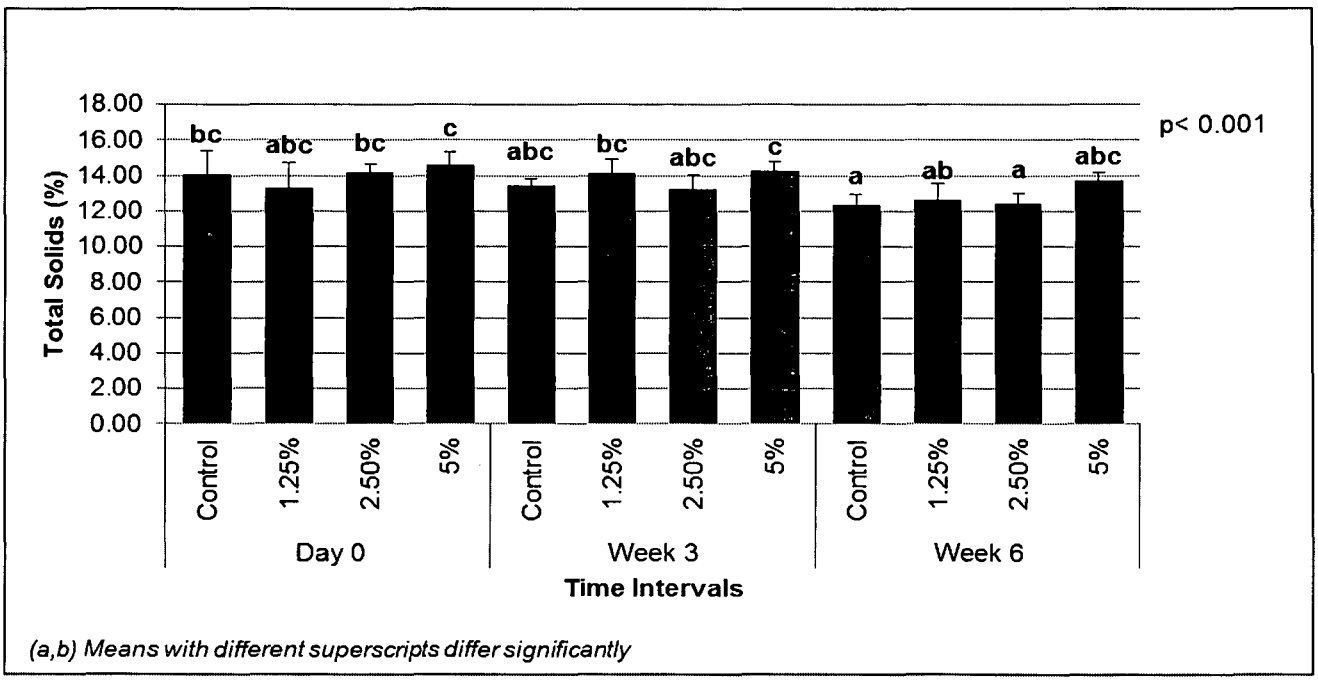


Figure 4.3 Yogurt total solids as affected by the storage time and the Tonalin® 60-WDP inclusion level.

After the six weeks storage time, a reduced TS content was observed for all the yogurt samples including the control samples (Fig 4.3). For the control and the 2.5 % Tonalin® this decline was statistically significant ($p < 0.001$). Similar results were also documented by Muhammad *et al.* (2009). Even for the yogurt with a 5 % Tonalin® inclusion level lower TS

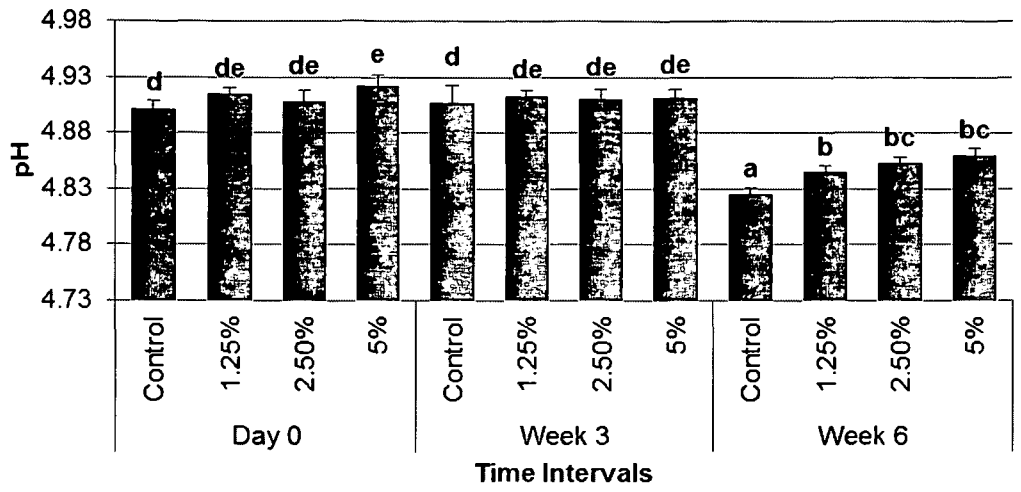
content was observed. The reduced levels for TS might have been due to the solubilization of part of the CLA microcapsule. Some of the non-fat solids contained in the yogurt might have been metabolized by the LAB. In a study by Laye *et al.* (1993) they reported that the decrease in TS content correlated with the decrease in the lactose concentration, as the lactose was metabolized by the LAB. This may explain the decline in TS for the control samples.

MICROBIAL AND CHEMICAL STABILITY

The yogurt fortified with Tonalin® showed slightly increased pH values compared to the control yogurt samples for all time intervals (Fig. 4.4). This increase was statistically significant ($p < 0.001$) in week 6. The increase in the pH might have been caused by the almost neutral pH of the Tonalin® product. The pH of the Tonalin® was influenced by the skim milk powder contained in the product, which had a pH of 6.6 to 6.7 (Jensen, 1995). The caseins of the microcapsule also had a strong buffering capacity (Shah *et al.*, 1995). The Tonalin® contained approximately 76 % of total fat. By increasing the inclusion level of Tonalin®, the additional amount of fat added to the yogurt also increased. Studies by Shaker *et al.* (2000) indicated that the pH increased slightly with increased fat content. This might have contributed to the slightly higher pH values for the yogurt with added Tonalin®.

The pH values for all the yogurt samples declined after six weeks of storage (Fig. 4.4). This decline was statistically significant ($p < 0.001$) for the control and 2.5 % Tonalin® inclusion level. The decrease in the pH of the yogurt samples may be attributed to the metabolic activities of the LAB in the yogurt culture (Shah *et al.*, 1995; Talwalkar & Kailasapathy, 2003; Obi *et al.*, 2010) and the continued activity of ß-galactosidase even at low temperatures (Muhammad *et al.*, 2009).

These results were in line with the findings of Shah (2000) who also reported decreases in pH values during the storage of commercial yogurts that contained *L. acidophilus* and *Bifidobacterium bifidum.* Yogurt starter cultures including *L. delbrueckii* spp. *bulgaricus* and *S. thermophilus* were active even at refrigerated temperature and could still produce small amounts of lactic acid by the fermentation of lactose which resulted in noticeable pH decreases (Shah *et al.*, 1995).



(a,b) Means with different superscripts differ significantly

Figure 4.4 pH of the yogurt as influenced by the storage time and the inclusion level of the Tonalin® 60-WDP.

Water activity (A_w)

Compared to the control samples, there was a statistically significant reduction in the A_w of the yogurt with the added Tonalin® (Fig. 4.5). The microcapsule of the Tonalin® combined strongly with water molecules which may have caused the reduction in the A_w. The microcapsule of the Tonalin® consists of skim milk powder and caseinates. Casein proteins have a very high water binding capacity (hydrophilic). The carbohydrates in the milk powder can also increase the amount of water bonded to the microcapsules (Singh & Ye, 2009) which explains the reduction in A_w in the yogurt supplemented with Tonalin®.

Lactic acid bacterial counts

Several reports have shown that the survival and viability of LAB are often low in yogurt (Gilliland & Speck, 1977; Schioppa *et al.*, 1981; Hull *et al.*, 1984; Shah *et al.*, 1995; Dave & Shah, 1997; Kailasapathy & Rybka, 1997; Shah, 2000; Lourens-Hattingh & Viljoen, 2001). The LAB counts were not significantly influenced by the Tonalin® inclusion levels. Storage time did however had a statistically significant ($p < 0.001$) effect on LAB counts (Fig. 4.6).

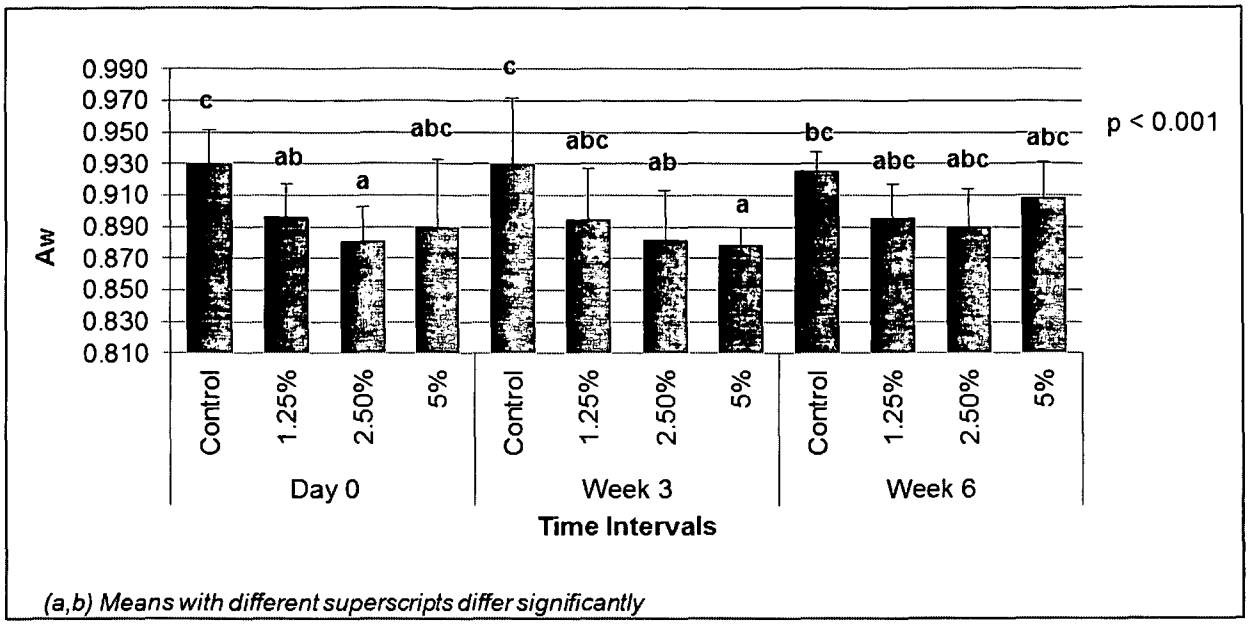


Figure 4.5 Water activity as affected by the Tonalin® inclusion in the yogurt.

The viability of the LAB declined over time (Tamime & Robinson, 1985) which explains the reduction in the total LAB counts over six weeks. From week 3 to week 6, there seemed to be a sharp decrease in the LAB counts, but if microbial analysis had also been done on week 4 and week 5, there would have been a more gradual decrease in the LAB up to week 6. The LAB counts of week 6 were significantly ($p < 0.001$) lower compared to week 0 and week 3 (Fig. 4.6).

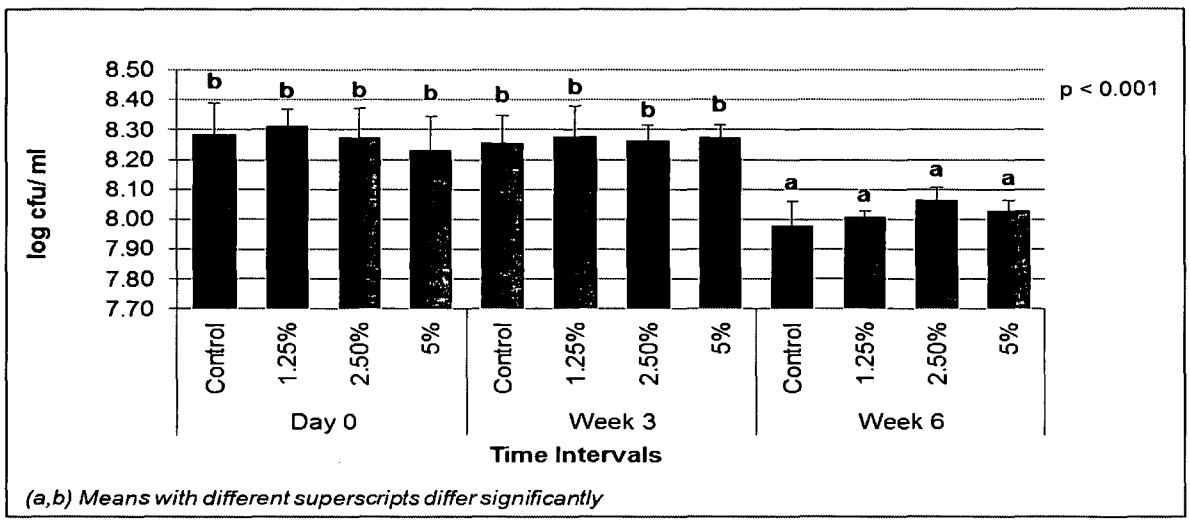


Figure 4.6 Total LAB after 6 weeks in the yogurt with different Tonalin® 60-WDP treatment levels.

The reduction in the LAB viability may be attributed to the sensitivity of the LAB to the lowered pH of the yogurt during storage (Gardiner *et al.*, 2000; Prasad *et al.*, 2003; Talwalkar & Kailasapathy, 2003; Capela *et al.*, 2007). This phenomenon of lowered pH is called "over acidification" (Shah *et al.*, 1995).

In another study by Ozer *et al.* (1998) they found that higher TS content of yogurt provided improved protection to the yogurt LAB from the lowering pH value. The decrease in the TS content and the decrease in the pH observed in this study possibly both contributed to the decrease in the viability of the LAB as the LAB were more exposed to the acidic environment as the TS levels decreased (Ozer *et al.*, 1998).

Viscosity

The viscosity of the yogurt increased significantly ($p < 0.001$) with increased Tonalin® inclusion levels. Even after six weeks storage, the viscosity of the yogurt samples that were fortified with 5 % Tonalin® was significantly ($p < 0.001$) higher than the control and the yogurt supplemented with 1.25 % Tonalin® (Fig. 4.7).

Increased amounts of Tonalin® resulted in increased levels of TS in yogurt. The solids form strong bonds with the free water which lead to an improved gel structure; therefore the viscosity will increase as the degree of syneresis is reduced. Similar results were found by Abrahamsen & Holman (1980), Tamime & Deeth (1980), Modler *et al.* (1983), Klupsch (1989) and Guinee & Mullins (1993) who found that the addition of dairy ingredients counteracted syneresis. In a study by Lucey *et al.* (1998), it was concluded that the best way to improve yogurt viscosity and to combat syneresis, was by increasing the solids content.

Casein can also increase viscosity. The calcium ions, which are an integral part of the casein micelles, become more soluble as the pH decrease. This may lead to improved firmness of the gel structure (Tamime & Deeth, 1980). The microcapsules of the Tonalin® contain high levels of casein that might have contributed to the increased viscosity values observed in this study.

The viscosity of the control yogurt samples declined significantly ($p < 0.001$) over the six weeks storage time (Fig. 4.7). This probably occurred due to syneresis. The continuous

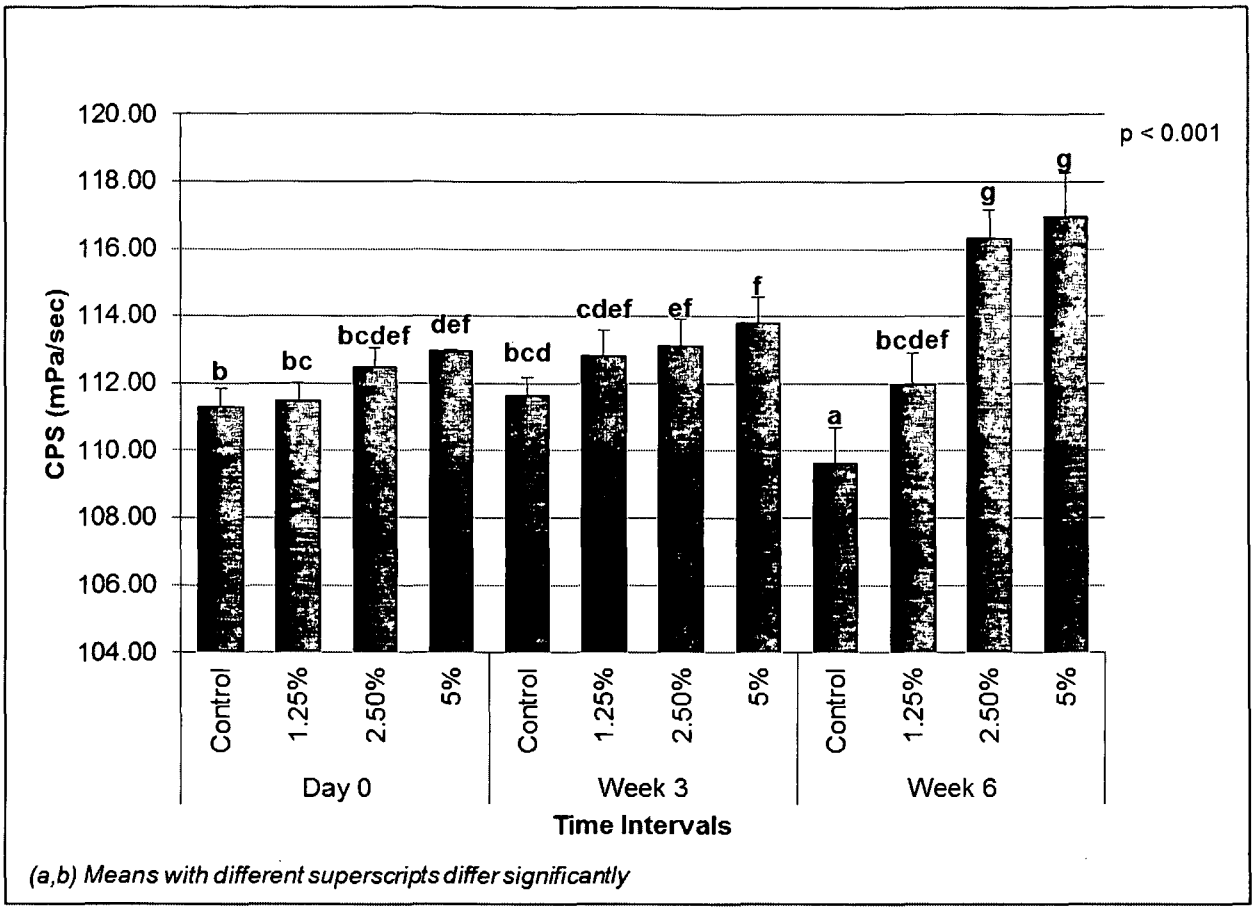


Figure 4.7 Yogurt viscosity in CPS as affected by the storage time and the Tonalin® 60-WDP inclusion level.

activity (acid production) of the LAB in the yogurt probably caused changes in the yogurt microstructure and hence affected the viscosity. The gel structure could also have been weakened by the lower pH resulting in water release, causing a decrease in the yogurt viscosity (Park *et al.*, 2002).

The viscosity of the 2.5 % and 5 % Tonalin® yogurt increased significantly ($p < 0.001$) over the six week storage period. From that can be deducted that yogurt syneresis can be limited with the addition of Tonalin®.

Oxidative stability

The oxidative stability of the yogurt samples were expressed as TBARS (thiobarbituric acid reactive substances). Usually it is expected that the TBARS of a product with an increased fat content would increase. In this experiment it was not the case. Fig. 4.8 illustrates that

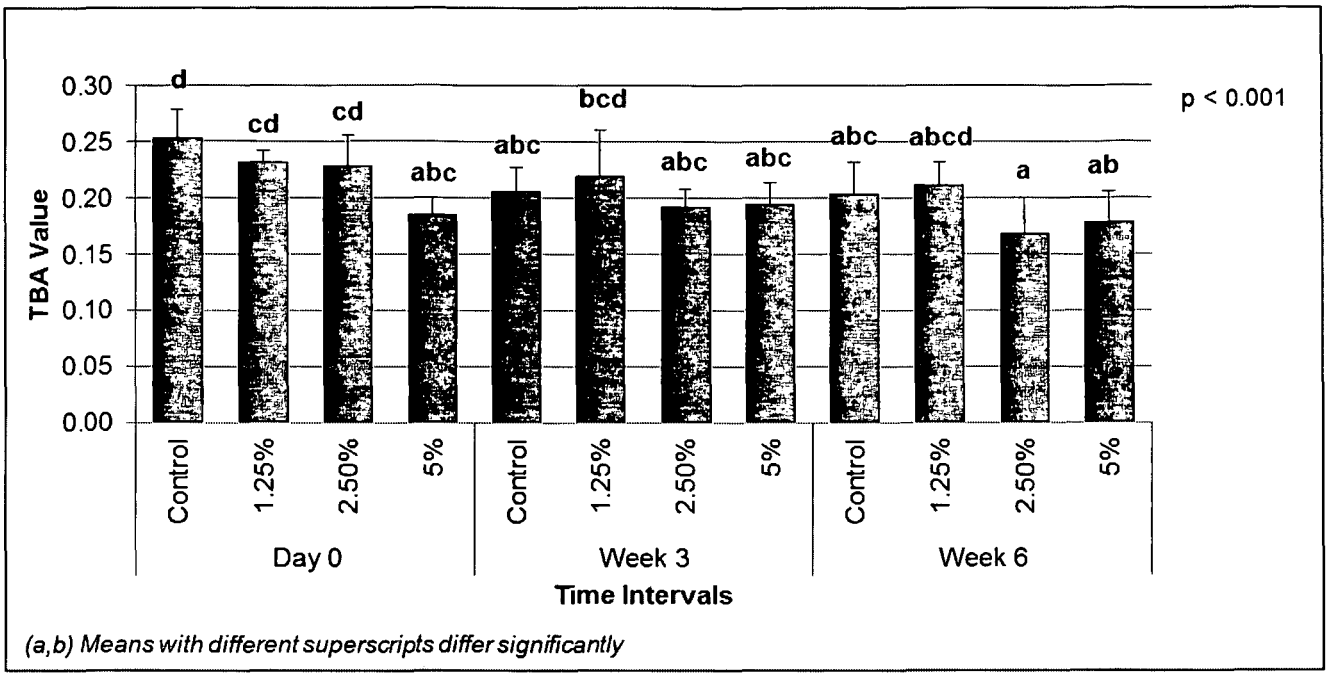


Figure 4.8 TBARS, indicating the degree of oxidation of the yogurt as influenced by storage time and the Tonalin® inclusion level.

the TBARS of the yogurt decreased with increased Tonalin® inclusion levels, despite the higher fat content. The decrease in the TBARS that was observed may be explained by the fact that CLA on its own is an antioxidant (Belitz & Grosch, 2008). The Tonalin® contained approximately 60 % CLA.

Viscosity also played a major role in the oxidation potential of the yogurt. Viscosity can affect oxidation by reducing the diffusion potential of pro-oxidative molecules. However, the role of viscosity on lipid oxidation of oil-in-water emulsions is not quite clear (Jacobsen *et al.*, 2001; Paraskevopoulou *et al.*, 2007). In a study by Jacobsen *et al.* (2001) the most viscous milk products were the least oxidized. In this study, the viscosity might have contributed to the lowered TBARS values as the more viscous (CLA supplemented) yogurt also had the lowest TBARS values.

The Tonalin® product that was used contained mixed tocopherols and ascorbyl palmitate which could also contribute to the higher oxidative stability of the yogurt (Cognis, 2004). The decrease in the TBARS with the inclusion of Tonalin® can also be due to the strong antioxidative properties of the milk proteins, particularly casein. Tonalin® contains high levels of casein (Cognis, 2004). Many studies proved that casein and whey proteins have strong anti-oxidative properties, as a result of their ability to bind transition metals and

scavenge free radicals (Hegenauer *et al.*, 1979; Taylor & Richardson, 1980; Allen & Wrieden, 1982; Ostdal *et al.*, 1996; Tong *et al.*, 2000; Chen *et al.*, 2003; Diaz *et al.*, 2003).

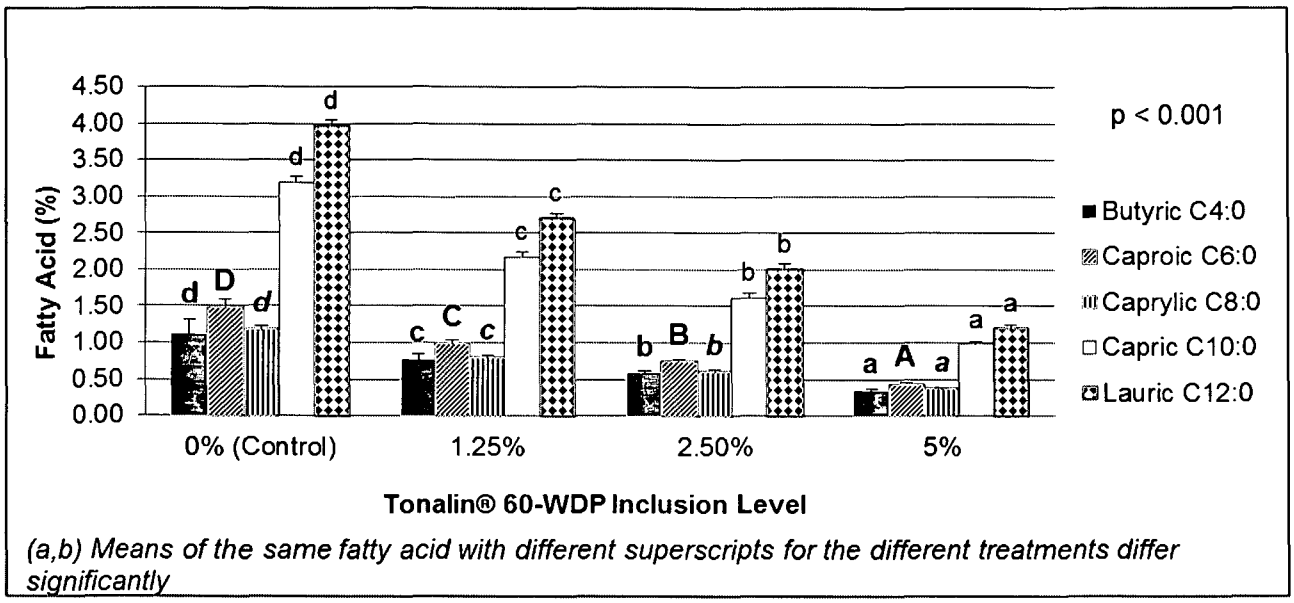
It is also possible that addition of Tonalin® improved the yogurt gel structure resulting in firmer yogurt. Once a gel is formed, the fat droplets are completely embedded in the protein network and are not easily accessible to be oxidized (Serra *et al.*, 2008).

The TBARS of all the yogurt samples, including the control, also decreased over the six weeks storage time (Fig. 4.8). In the case of the control this decline was significant ($p < 0.001$). This may be due to the liberation of the antioxidant peptides with high radical scavenging ability from the casein and whey proteins naturally contained in the yogurt (Virtanen *et al.*, 2007). In a study by Corredig & Dalgleish (1996) they found that the lipid droplet membrane in yogurt was likely to contain more whey protein than for example the lipid droplet membrane in pasteurized milk, due to the higher pasteurization temperature in yogurt manufacturing. The presence of the protein, ß-lactoglobulin also reduced oxidation. Milk exposed to higher temperatures, showed an increased content of this main whey protein, ß-lactoglobulin (Sørensen *et al.*, 2007).

The LAB contained in the yogurt also exhibit natural anti-oxidative properties. These anti-oxidant activities are believed to originate from cellular lysis and the release of intracellular metabolites (Lin & Yen, 1999a; Lin & Yen 1999b; Saide & Gilliand, 2005). Conformational changes in the proteins in yogurt due to the lower pH, the gel structure and the microbial activity in the yogurt may decrease the diffusion of potential pro-oxidative molecules in the yogurt and it might also have decreased the availability of the transition metal ions to oxidation reactions (Let *et al.*, 2007).

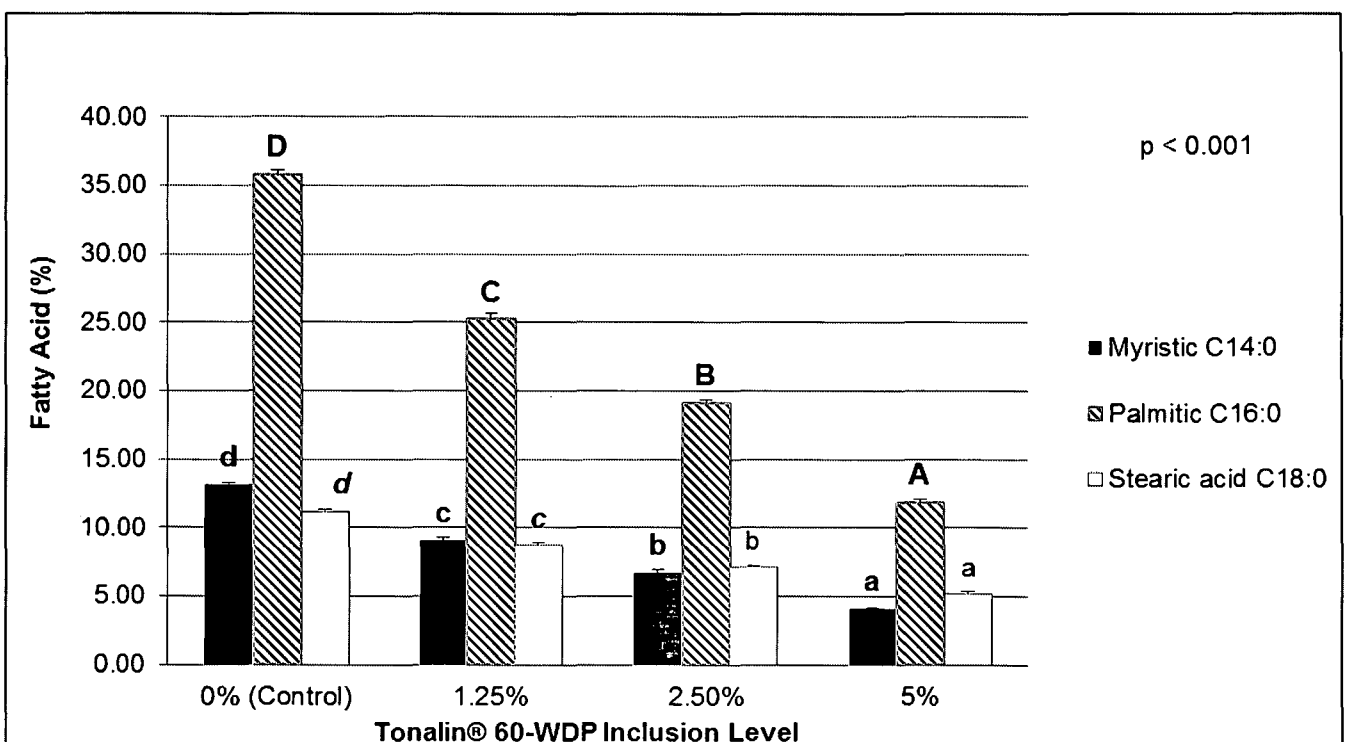
FATTY ACID COMPOSITION

The short and medium chain as well as long chain SFA's decreased significantly ($p < 0.001$) with increased Tonalin® inclusion levels (Fig. 4.9 and Fig 4.10). The decrease in the SFA content with increased Tonalin® levels was due to dilution because of the high PUFA's content of the Tonalin® (Chouinard *et al.*, 1999). In a study by Cruz-Hernandez *et al.* (2007) they also have found that the greatest changes were observed in the SFA's when PUFA's were supplemented.



(a,b) Means of the same fatty acid with different superscripts for the different treatments differ significantly

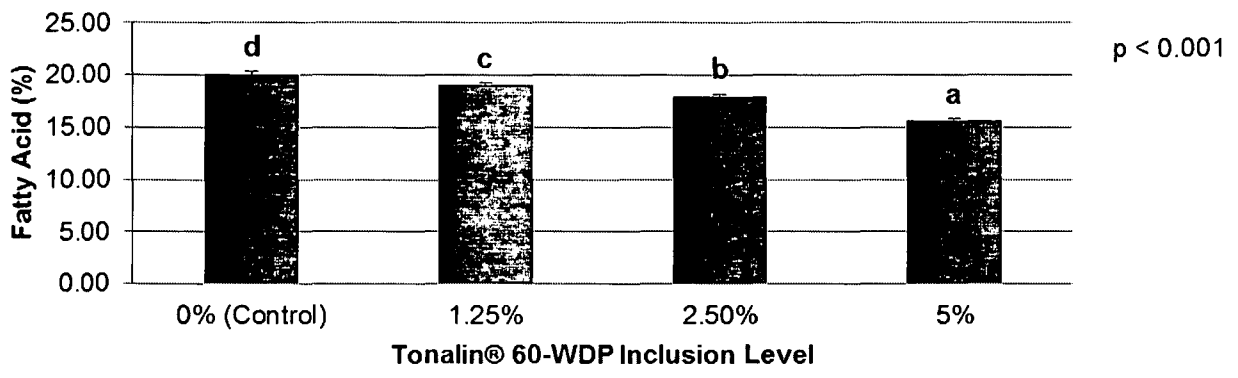
Figure 4.9 Short and medium chain SFA's as affected by the Tonalin® 60-WDP inclusion level.



(a,b) Means of the same fatty acid with different superscripts for the different treatments differ

Figure 4.10 Long chain SFA's as affected by the Tonalin® 60-WDP inclusion level.

The MUFA's of the yogurt decreased significantly ($p < 0.001$) with increased Tonalin® inclusion level (Fig. 4.11). As in the case of the SFA's, the MUFA's of the yogurt decreased with increased Tonalin® inclusion levels because of as a result of the high concentration of PUFA's in the Tonalin® product. The Tonalin® contains at least 60 % PUFA's and therefore the ratios for the SFA's and MUFA's will decrease with increased Tonalin® levels (Chilliard *et al.*, 1991; Boylston & Beitz, 2002; Cognis, 2004).



(a,b) Means with different superscripts between the treatments differ significantly

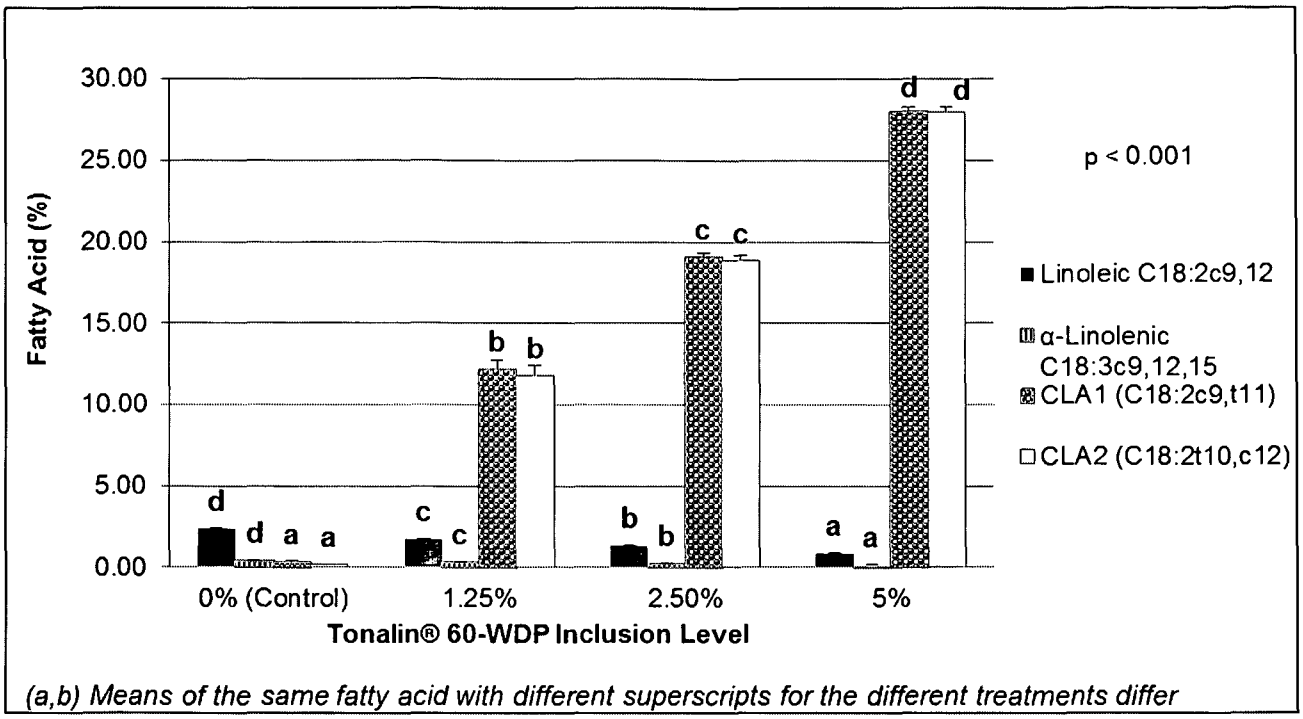
Figure 4.11 MUFA's as affected by the Tonalin® 60-WDP inclusion level.

With increased Tonalin® inclusion levels, the concentration of the LA and the α-linolenic acid of the yogurt decreased significantly ($p < 0.001$) (Fig. 4.11). The LA and the α-linolenic acid that were detected in the control yogurt, were at levels naturally occurring in yogurt and the concentration of these two PUFA's decreased significantly ($p < 0.001$) due to the high concentration of the other PUFA's in the Tonalin® fortified products (Chilliard *et al.*, 1991; Boylston & Beitz, 2002; Cognis, 2004).

A drastic increase in CLA1 and CLA2 occurred with increased Tonalin® inclusion levels (Fig. 4.12) due to the high levels of these two CLA isomers in the Tonalin® product (Cognis, 2004).

Actual CLA content

The total CLA per 100 g of yogurt increased with increased Tonalin® inclusion levels (Fig. 4.13). The high concentration of total CLA contained in the Tonalin® product used for the



(a,b) Means of the same fatty acid with different superscripts for the different treatments differ

Figure 4.12 PUFA's as affected by the Tonalin® inclusion level

fortification of the yogurt, caused the increase in the total CLA concentration of the fortified yogurt (Cognis, 2004).

The highest level of CLA that was obtained in 100 g of yogurt was with a 5 % Tonalin® inclusion level (Fig. 4.13). The concentration of CLA in in the yogurt with a 5 % Tonalin® inclusion level was approximately 2.8 g. The aim was to reach an amount of 3 g of total CLA in 100 g of yogurt. The reason for the slightly lower total CLA concentration than what was aimed for might have been the fact that the total CLA concentration of the Tonalin® varied between 58 % and 62 % in the product itself (Cognis, 2004).

A concentration of approximately 3 g of total CLA per 100 g of yogurt as was obtained with a 5 % Tonalin® inclusion, represents 100 % of the RDA of CLA (Fig. 4.13). In other words, a consumer would receive 100 % of the CLA needed per day to reach maximum health benefits, with the consumption of only 100 g of this CLA fortified yogurt.

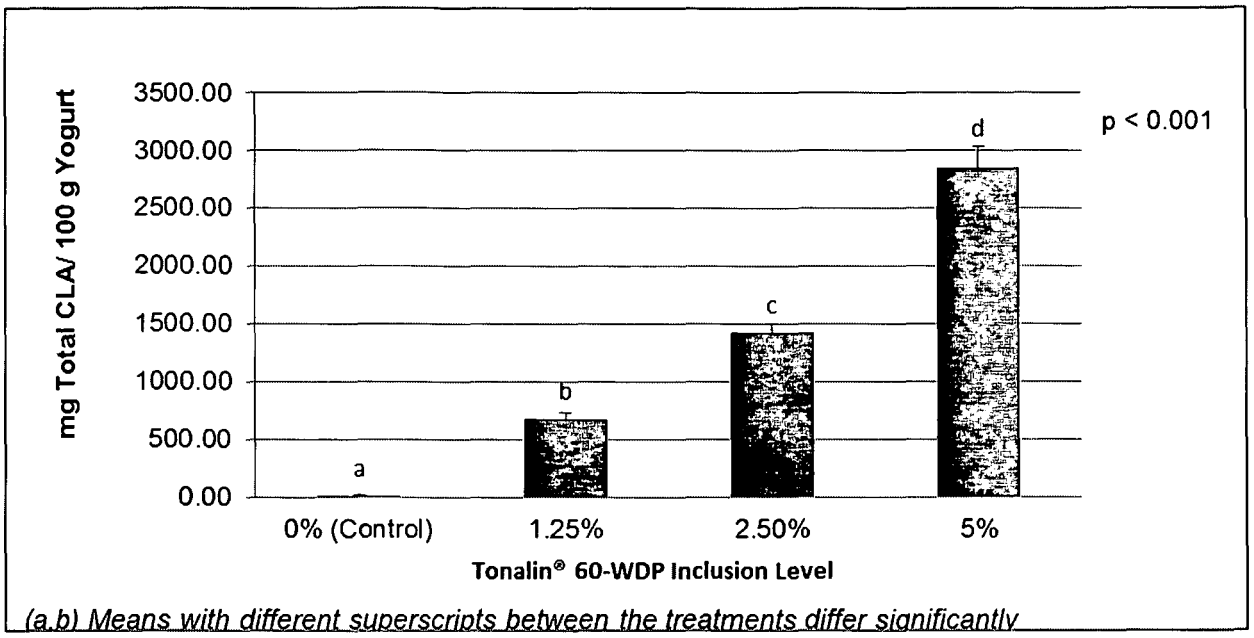


Figure 4.13 Total CLA in 100 g of yogurt as affected by the Tonalin® inclusion level.

SENSORY ANALYSIS

An untrained consumer panel tasted the strawberry yogurt contained in white tubs which were randomly numbered. The demographic profile of the panel is depicted in Table 4.2. The consumers appointed scores for the attributes of taste, mouthfeel, aftertaste and overall liking for the yogurt with all four treatment levels of Tonalin®.

Table 4.2 Demographic Profile of Consumer Panel

Gender:	% of Total	Age:	% of Total
Female	75	**< 20**	4
Male	25	**20-29**	43
		30-39	12
		40-49	13
		50-59	21
		>60	7

The scores for the two yogurt batches with the lowest Tonalin® inclusion levels (control and 1.25 %) differed significantly ($p < 0.001$) from the scores of the yogurt with the highest Tonalin® inclusion levels (2.5 % and 5 %) (Table 4.3).

Although the yogurt with the two lowest Tonalin® inclusion levels were preferred above the two higher inclusion levels by the consumer panel, the difference between the scores for

Table 4.3 Analysis of Variance (ANOVA) of Consumer Panel Data

Tonalin® 60-WDP Inclusion Level	0.00 %	1.25 %	2.50 %	5.00 %	Significance Level
Taste	7.27 ± 1.30^{b}	7.44 ± 1.33^{b}	6.28 ± 1.76^{a}	6.31 ± 1.65^{a}	$p < 0.001$
Mouthfeel	7.38 ± 1.29^{b}	7.48 ± 1.26^{b}	6.55 ± 1.63^{a}	6.15 ± 1.59^{a}	$p < 0.001$
Aftertaste	6.63 ± 1.70^{b}	6.97 ± 1.64^{b}	5.82 ± 1.94^{a}	5.55 ± 1.76^{a}	$p < 0.001$
Overall liking	7.21 ± 1.36^{b}	7.46 ± 1.31^{b}	6.41 ± 1.66^{a}	6.35 ± 1.43^{a}	$p < 0.001$

(a,b) Means with different superscripts in the same row differ significantly

the two preferred yogurt batches and the yogurt with the two highest Tonalin® inclusion levels, were small (Table 4.3). The scores for the yogurt of all four the Tonalin® inclusion levels, were between a score of 6 (like slightly) to a score of 8 (like very much), which means that none of the yogurt treatments were disliked by the consumer panel.

The findings of the panel are also summarized in Fig. 4.14. This figure clearly illustrates that the difference in sensory scores between the more preferred and least preferred samples were very small. The possibility does exist that the strawberry flavour partially disguised the actual taste of the Tonalin® and hence improved the overall consumer acceptability. In a study by (Campbell *et al.*, 2003) they have found that chocolate flavoured CLA fortified milk obtained higher acceptability scores by consumers than unflavoured milk with the same elevated CLA levels.

Although the yogurt from this study was accepted by consumers as demonstrated by the sensory scores, it is possible that the overall acceptability of the CLA fortified yogurt would not be of any concern to the consumers if they were informed on the health benefits of the product. The benefits of the yogurt with elevated CLA levels can be defined as being credence characteristics, which means that it is characteristics that cannot be discerned by normal use, e.g. the healthiness of a product (Peng *et al.*, 2006). In studies by Bech-Larsen & Grunert (2003) they suggested that the health and nutritional information affected the consumers' attitudes towards a functional food product. In general consumers are willing to pay a premium for a functional food product regardless the possibility of a slight taste and price difference (Campbell *et al.*, 2003; Jimenez *et al.*, 2008).

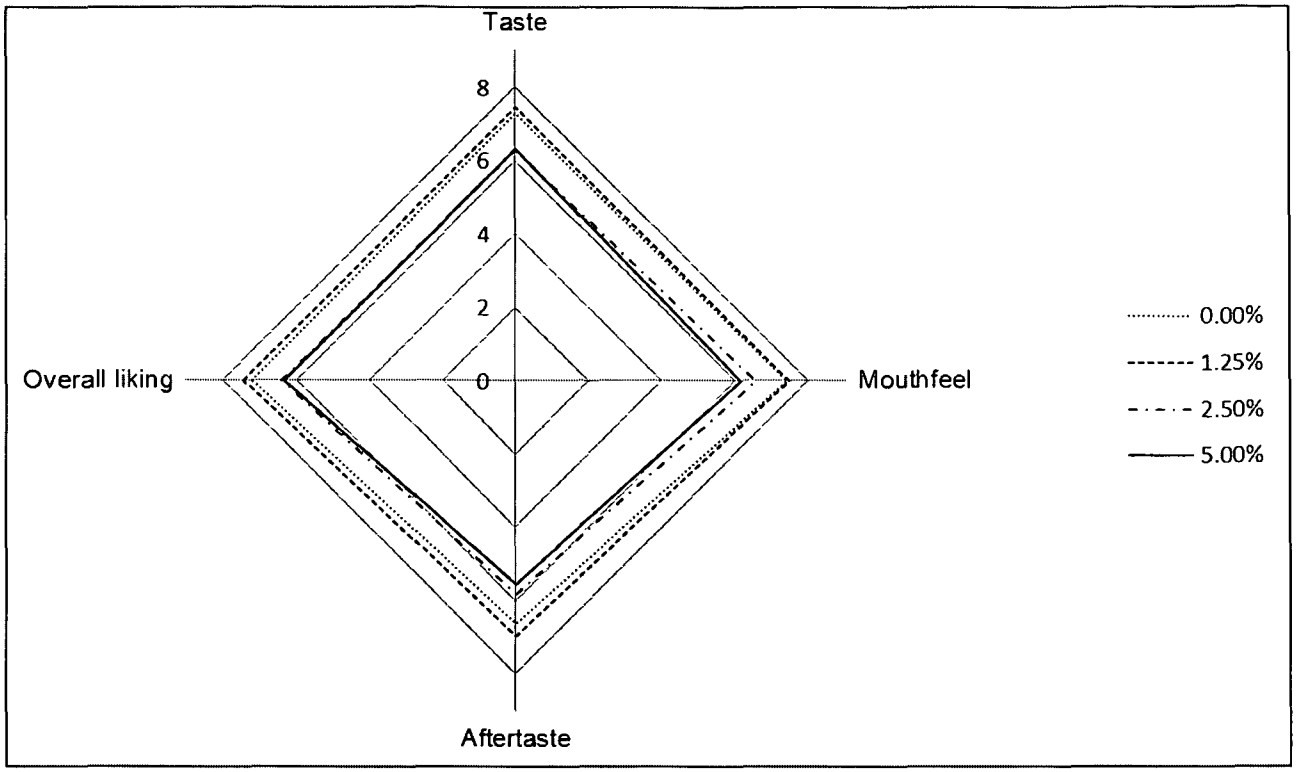


Figure 4.14 Consumer scores for taste, mouthfeel, aftertaste and overall liking as affected by the Tonalin® inclusion level.

4.4 CONCLUSION

In this study it was found that the addition of the Tonalin® to yogurt in order to elevate the CLA concentration of the yogurt also resulted in an increase in the fat content of the yogurt. This may be regarded as negative by most consumers. Therefore it is necessary to inform the consumers that the additional fat is mainly CLA. CLA is handled completely differently by the human body than other fatty acids and would not have a negative impact on health. The FA ratios were also altered upon the addition of Tonalin®. The PUFA's increased which caused the reduction in the percentage SFA's. This is also a positive effect obtained with the fortification of yogurt with synthetic CLA, since SFA's are negatively associated with human health.

The total solids content of yogurt was also increased with the addition of the Tonalin®. This may be regarded as positive as the yogurt is "protected" against the effects of syneresis by the contribution of Tonalin® to the higher solids content. The presence of the LAB in the yogurt is important due to the positive health benefits associated with the live cultures. Addition of the Tonalin® did not affect the LAB counts. Thus by improving another health

attribute of yogurt, the existing health benefits of yogurt would not be destroyed. The oxidative stability of the yogurt during a six week shelf-life period was also improved with the addition of Tonalin®. The yogurt was accepted by the consumer panel regardless of the level of Tonalin® inclusion. This is a major positive factor, as an unacceptable product would not sell. Even the highest Tonalin® inclusion to the yogurt, was acceptable. The full recommended daily amount of CLA can thus be reached by consuming only 100 g of yogurt.

Elevating the yogurt CLA levels with the addition of a synthetic CLA product, such as Tonalin® creates a more stable yogurt product with very good health benefits. Fortification of yogurt with synthetic CLA such as Tonalin® may therefore be considered for the development of a new functional dairy food product.

A number of positive effects of the synthetic CLA fortification on the yogurt were reported in this study. Nutraceuticals are foods with perceived medicinal or health benefits that may prevent, ameliorate or cure a disease. Taking the definition of a nutraceutical into consideration, the supplementation of a synthetic CLA product changes yogurt into a true nutraceutical.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

The major CLA isomer contained in natural products is the C18:2c9t11 (CLA1) isomer, also known as rumenic acid (RA). This isomer is predominantly produced by ruminants during biohydrogenation of LA by rumen bacteria (Bauman *et al.*, 2000). Although CLA2 is not as abundant as CLA1 in natural food products, these two CLA isomers are the most biologically active CLA isomers (Ha *et al.*, 1987; Ha *et al.*, 1989; Ip *et al.,* 1991; Schultz *et al.*, 1992).

Several studies using animal models, suggested that CLA1 was responsible for anti-carcinogenic, growth-promoting as well as anti-atherogenic properties (Khanal, 2004; Lee *et al.*, 2005). In a study by Pariza (2004) it was suggested that CLA2 has lean body mass-enhancing properties. It was suggested that if these advantages were transferred to humans, increased CLA consumption could have a positive effect on the nutritional value of food that contains CLA (Precht & Molkentin, 2000).

Ruminant milk is the richest natural source of CLA (Ha *et al.*, 1989; Chin *et al.*, 1992; Shanta *et al.*, 1995). Concentrations of CLA in milk and milk products were reported to vary markedly between countries and specific products (Precht & Molkentin, 2000). Several techniques have been studied to increase the CLA content in dairy products. After it was found that some fermented dairy products contain higher amounts of CLA than non-fermented dairy products, some authors suggested that with the use of certain starter cultures, the CLA concentration of the final food product could potentially be increased (Chin *et al.*, 1992; Ha *et al.*, 1989; Lin *et al.*, 1995; Lin *et al.*, 1999; Shanta *et al.*, 1992).

Results from other researchers that studied CLA production by lactic acid bacteria (LAB) were however contradictory as some authors found significant increases in CLA levels and some authors detected no CLA production by LAB (Gnädig *et al.*, 2004). Additional experiments need to be done to fully examine the possibility of increasing CLA levels in dairy products. Studies on the effects of CLA fortification on product stability during

production and storage should support the development of consumer-acceptable and enhanced dairy foods of proven quality (Rodríguez-Alcalá & Fontecha, 2007).

The first aim of this study was to evaluate the potential CLA production by the three most frequently used yogurt starter cultures in the South African yogurt industry with the addition of LA sources. Many studies have already been published in this specific research area, but most of them investigated selected single bacterial strains under controlled conditions in laboratory media (Sieber *et al.*, 2004). In this study the ability of the three mixed cultures in a milk medium used for yogurt manufacturing was investigated.

The linoleic acid (LA) and sunflower oil (SFO) as LA sources were added to the appropriate milk samples after which the milk was inoculated with the specific starter culture. A pH of 4.6 was reached after a fermentation period of approximately 5 hours. All the samples were then frozen for further analysis. The addition of the lipid sources could change the fatty acid (FA) composition of the yogurt (Kim & Liu, 2002). The degree of the possible changes was investigated in the current part of the study.

The total fat content of the yogurt were influenced by the initial fat level of the milk, the LA source and the type of culture that was used. The full cream yogurt had an average total fat content significantly ($p < 0.001$) higher than the average total fat content of the fat free yogurt, due to the natural higher fat content of the full cream milk that was used for the manufacturing of the full cream yogurt (Belitz & Grosch, 2008). The addition of the LA and SFO to the yogurt led to a significantly ($p < 0.001$) higher total fat content compared to the full cream control and fat free yogurt control samples. Variations exist between the metabolic activities of the different starter cultures (Urbach, 1995), which might have led to the different total fat levels that occurred between the yogurt samples. According to (Urbach, 1995) the yogurt starter cultures metabolize some of the fatty acids and this leads to changes in the total fat content. In studies by Bonczar *et al.* (2002) and Belitz& Grosch (2008) it was found that an inverse association existed between the total fat content and the total solids (TS) and moisture content. This was also confirmed in the results from this study. The yogurt with the highest total fat content had the lowest total moisture and TS content.

The FA profiles of the yogurt samples were significantly ($p < 0.001$) changed by the LA source and the fat level of the yogurt. The full cream yogurt samples had a significantly (p

< 0.001) higher saturated fatty acid (SFA) content compared to the fat free yogurt samples. This can be attributed to the natural high SFA content contained in milk fat and the full cream yogurt samples (Belitz & Grosch, 2008). With the addition of the LA, a lower SFA content and higher polyunsaturated fatty acid (PUFA) content was observed, due to the high amount of PUFA's contained in the added LA (Chilliard *et al.*, 1991; Jenkins, 1993; Boylston & Beitz, 2002). This trend was observed in the full cream and fat free yogurt samples. A difference in the FA profile even between the LA sources was obtained. It was found that in the full cream and fat free yogurt samples with added SFO, a higher PUFA content occurred similar to the yogurt with added LA. In order to obtain similar LA concentration, more SFO was added to the yogurt; resulting in higher PUFA's in the yogurt with added SFO (Chilliard *et al.*, 1991; Jenkins, 1993; Boylston & Beitz, 2002).

Conjugated linoleic acid is also a group of PUFA's (Belitz & Grosch, 2008) and therefore the actual amount of CLA was not only influenced by the fat level of the yogurt, but also by the type of LA source and the effect of the LA source on the starter culture. The full cream yogurt samples had a higher CLA content compared to the fat free yogurt samples. This may be attributed to the higher total fat content of the full cream yogurt as CLA forms part of the total fat content (Belitz & Grosch, 2008). The samples with the added LA sources had higher actual CLA contents than the control yogurt samples, which also proves the suggestion by Sieber *et al.* (2004) that additional LA is needed for the conversion to CLA by the LAB. The ability of the starter cultures to convert LA to CLA fluctuated significantly ($p < 0.001$) due to the differences in the metabolic and enzyme LA isomerase activity between starter cultures (Urbach, 1995). The highest CLA content in the full cream yogurt was obtained with the addition of SFO and the use of the YC-X11 starter culture. The highest CLA content that was obtained in the fat free yogurt was with the addition of LA and the use of the YC-180 starter culture.

Although elevated CLA levels were obtained with natural conversion by the starter cultures, the amount was still very low. This has led to the second part of the study of which the aim was to fortify yogurt with synthetic CLA. The experiment was designed in such a way that yogurt with elevated CLA levels represented 25 %, 50 % and 100 % of the RDA of CLA in only 100 g (1 serving size) of yogurt.

Conjugated linoleic acid is a group of FA's and hence forms part of the total fat content of the product (Belitz & Grosch, 2008). The synthetic CLA product, Tonalin® 60-WDP that

was used, contained approximately 60 % CLA and approximately 76 % total fat (Cognis, 2004). With increased Tonalin® inclusion levels, increases in total fat content was observed. The Tonalin® also contained carbohydrates and proteins in the microcapsule that protected the CLA. This also explains the increased TS content that occurred with increased Tonalin® inclusion levels. Although increased TS contents were observed for the yogurt with increased Tonalin® inclusion levels, the TS content of the yogurt decreased during the six weeks storage time. According to Muhammad *et al.* (2009) this is due to the fact that some of the solids might have been metabolized by the LAB contained in the yogurt.

Generally the pH of yogurt decrease over time due to residual metabolic activities of the LAB (Shah *et al.*, 1995; Talwarker & Kailasapathy, 2003; Obi *et al.*, 2010). This was also observed in the results obtained from these experiments. This post-acidification during storage is due to the ß-galactosidase (ß-gal) which is still active between 0°C and 5°C, resulting in the formation of D-glucose and D-galactose. The glucose is further fermented by LAB to lactic acid, causing the pH to decrease to less than 4.2. (Muhammad *et al.*, 2009). With the addition of the Tonalin®, there was a slight increase in the pH and it could be suggested that the addition of the Tonalin® protects the yogurt to a certain extent from the "over-acidification" which may influence the viability of the probiotic cultures.

A reduction in the water activity of the yogurt with increased Tonalin® inclusion levels was observed. The high carbohydrates contained in the microcapsule of the Tonalin® binds the free water which probably caused the reduction in the water activity (Singh & Ye, 2009). With the changes in the water activity and the pH, it was also expected to find changes in the LAB counts as the viability of the LAB may be influenced by these factors (Gilliland & Speck, 1977; Schioppa *et al.*, 1981; Hull *et al.*, 1984; Shah *et al.*, 1995; Dave & Shah, 1997; Kailasapathy & Rybka, 1997; Shah, 2000; Lourens-Hattingh & Viljoen, 2001). With the addition of Tonalin® to the yogurt, no significant effects on the viability of the LAB were detected, but the total LAB counts were significantly ($p < 0.001$) influenced by the storage time. The viability of the LAB decreased over the six weeks storage time. According to other studies (Gardiner *et al.*, 2000; Prasad *et al.*, 2003; Talwalkar & Kailasapathy, 2003; Capela *et al.*, 2007) the main reason for the declined viability of the LAB was due to their sensitivity to the more acidic environment. With all these changes that were observed, it was not surprising to find changes in the yogurt viscosity. The viscosity of the yogurt samples tend to increase with increased Tonalin® inclusion levels. This was due to the

increased TS content caused by the additional Tonalin® that was added. The solids formed strong bonds with the water, which in turn cased the viscosity to increase (Abrahamsen & Holman, 1980; Tamime & Deeth, 1980; Modler *et al.*, 1983; Klupsch, 1989; Guinee & Mullins, 1993). While increased viscosity values were detected with increased Tonalin® inclusion levels, the viscosity of the control yogurt samples decreased over the six weeks storage time. The lowered pH caused the weakening of the yogurt gel structure and this led to a lower viscosity (Park *et al.*, 2002). The addition of Tonalin® to the yogurt, protected the yogurt from the effects of syneresis.

Usually with increased PUFA levels found with the addition of Tonalin®, it is expected that the oxidative stability of the product would decrease. In this experiment it was not the case. With the addition of Tonalin®, the TBARS decreased. The higher oxidative stability of the yogurt with Tonalin® was probably due to the fact that CLA on its own is an antioxidant and the Tonalin® also contained added antioxidants (Belitz & Grosch, 2008). Although the oxidative stability of the yogurt samples initially increased with the addition of Tonalin®, a decline in the TBARS for all the yogurt samples, including the control samples, were observed over the six weeks storage time. The main reason for the improved oxidative stability may be attributed to the antioxidative nature of the LAB contained in the yogurt and the protective effect of the natural yogurt proteins against oxidation (Hegenauer *et al.*, 1979; Taylor & Richardson, 1980; Allen & Wrieden, 1982; Ostdal *et al.*, 1996; Tong *et al.*, 2000; Chen *et al.*, 2003; Diaz *et al.*, 2003).

With the increased PUFA content caused by the addition of the Tonalin®, the changes in the FA profile of the yogurt were not surprising. The SFA and the mono-unsaturated fatty acid (MUFA) content of the yogurt decreased with increased Tonalin® inclusion levels due to the PUFA content of the product and therefore the PUFA content of the yogurt also increased with increased Tonalin® inclusion levels (Chouinard *et al.*, 1999; Cruz-Hernandez *et al.*, 2007). Conjugated linoleic acid forms part of the PUFA's contained in the Tonalin® and therefore changes in the actual CLA content were observed in the fat fraction of the yogurt. The CLA content of both the CLA isomers increased with increased Tonalin® inclusion levels. These two isomers existed in a 50:50 ratio in the Tonalin® product which contained approximately 60 % of total CLA. The increased actual CLA content caused the CLA content in the yogurt to increase as well. With the 5 % Tonalin® inclusion, 100 % of the RDA for CLA was contained in only 100 g of yogurt, which is equal to one portion. Although the CLA content of the yogurt was successfully elevated, with no adverse effects

on the yogurt's chemical and physical properties, the results from the sensory analysis would determine the actual potential for the development of the CLA fortified yogurt.

Slight differences were detected in the scores for the yogurt samples with the different Tonalin® treatment levels. Although the control yogurt and the yogurt with the 1.25 % Tonalin® inclusion were more preferred by the consumer panel, the scores were not much higher than the scores obtained for the yogurt with the two highest Tonalin® inclusion levels (2.5 % and 5 %). The yogurt with all four the Tonalin® treatment levels obtained scores between "6" and "8" which means that the inclusion of Tonalin® at any of the four treatment levels, were accepted by the consumer panel.

With the positive results from the sensory evaluation of the CLA fortified yogurt, the possibility to develop CLA fortified yogurt in the industry, becomes even greater. With the addition of Tonalin® to the yogurt, not one of the yogurt samples were disliked and the Tonalin® inclusion had only positive effects on the stability, chemical and physical properties of the yogurt. With all these results it can be concluded that the full potential of yogurt and other dairy products have not been discovered yet. With thorough research, the possibility exists that yogurt could marketed in the future as a true nutraceutical.

FUTURE RESEARCH

Surveys must be performed to gather more information on the consumer's attitudes towards the idea of CLA fortified yogurt. This can only be done after complete calculations were carried out on the effect of synthetic CLA addition to yogurt on the final yogurt cost. Yogurt with different inclusion levels of synthetic CLA should also be evaluated by a trained sensory panel. More research can also be done on the type of yogurt flavour that disguises the CLA taste most effectively. Further research on CLA fortification of cheese is also a suggestion. Cheese contains higher levels of fat than yogurt and the impact of CLA fortification on the flavour of the product might be less.

CHAPTER 6

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CHAPTER 7

SUMMARY

Conjugated linoleic acid has proven beneficial health properties. Approximately 18 different conjugated linoleic acid isomers exist. Two of these isomers (C18:2c9t11 and C18:2t10c12) are the most biologically active forms of conjugated linoleic acid. Results from several studies over the past few years confirmed the health benefits of conjugated linoleic acid. These benefits include anti-carcinogenic, anti-adipogenic, anti-atherogenic, anti-diabetogenic and anti-inflammatory properties. Conjugated linoleic acid consumption can also significantly decrease body fat mass without significantly altering the body weight. To reach maximum health benefits, the recommended dietary allowance for conjugated linoleic acid is in the range of 3 to 3.5 g per day. This value may vary among individuals. This is much more than the amount of conjugated linoleic acid present in dairy products, despite the fact that dairy products are the richest natural source. Approximately 45 kg of conventional milk must be consumed daily to supply enough conjugated linoleic acid for maximum health benefits. The aim of this study was therefore to increase the conjugated linoleic acid content of yogurt to levels closer to the recommended dietary allowance.

The first approach was to naturally increase full cream and fat free yogurt conjugated linoleic acid levels using selected starter cultures and linoleic acid sources. Three most frequently used commercial yogurt starter cultures (YC-180, YC-X11 and ABT-5) were used. Linoleic acid and sunflower oil as were used as linoleic acid sources. Linoleic acid and sunflower oil were added to full cream yogurt and fat free yogurt to supply linoleic acid in a concentration of 1 mg/ml in the media. The full cream control yogurt had a significantly ($p < 0.001$) higher total CLA content than the fat free control yogurt. Fat free yogurt with linoleic acid had a slightly higher CLA content than the fat free yogurt control and fat free yogurt with sunflower oil. The highest CLA concentration in the full cream yogurt was obtained with starter culture YC-X11 and the highest CLA concentration in fat free yogurt was obtained with starter culture YC-180. Approximately 15 kg of this naturally CLA fortified yogurt however will still need to be consumed on a daily basis to achieve maximum health benefits. This is closer to the RDA than for conventional yogurt, but still not nearly sufficient.

The second part of the study was therefore designed to increase CLA levels in yogurt by direct fortification with synthetic CLA (Tonalin® 60-WDP). The Tonalin® was added in four treatment levels (0 %, 1.25 %, 2 % and 5 %) and the influence of the fortification over a six-week storage period was evaluated. The total CLA per 100 g of yogurt increased with increased Tonalin® levels. The highest level of CLA that was obtained in 100 g of yogurt was with a 5 % Tonalin® inclusion level yielding approximately 2.8 g CLA. This means that a 5 % Tonalin® inclusion represents 100 % of the RDA of CLA. In other words, a consumer would receive 100 % of the CLA needed per day to achieve maximum health benefits, with the consumption of only 100 g of yogurt of this CLA fortified yogurt. Storage time had no significant ($p < 0.001$) influence on the total CLA content of the yogurt. Sensory evaluation on the yogurt with the four Tonalin® inclusion levels was done. The yogurt with the two lowest Tonalin® inclusion levels (0 % and 1.25 %) obtained significantly higher scores than the yogurt with the two highest Tonalin® inclusion levels (2.5 % and 5 %). The scores for the yogurt of all four the Tonalin® inclusion levels, were between a score of 6 (like slightly) to a score of 8 (like very much), which means that not one of the yogurt batches with any treatment level of Tonalin® were disliked by the consumer panel.

It was established that CLA fortification of yogurt is possible. Natural CLA production by yogurt starter cultures increased the CLA levels, but CLA levels equal to or close to the RDA were obtained with direct CLA fortification. Therefore direct fortification with synthetic CLA may be considered a more realistic approach for the development of a new functional dairy food product.

Keywords: Conjugated linoleic acid, linoleic acid, sunflower oil, fortification, starter culture, synthetic.

CHAPTER 8

OPSOMMING

Daar is bewys dat inname van gekonjugeerde linoleïensuur 'n aantal voordelige gesondheids eienskappe inhou. Ongeveer 18 verskillende gekonjugeerde linoleïensuur isomere bestaan. Twee van hierdie isomere (C18: 2c9t11 en C18: 2t10c12) is die mees biologies aktiewe vorme van gekonjugeerde linoleïensuur. Die resultate van verskeie studies het die gesondheidsvoordele van gekonjugeerde linoleïensuur bevestig. Om maksimum gesondheidsvoordele te bekom, is die aanbevole daaglikse inname (ADI) van gekonjugeerde linoleïensuur ongeveer 3 tot 3.5 g. Dit is baie meer as die hoeveelheid gekonjugeerde linoleïensuur in suiwelprodukte, ten spyte van die feit dat suiwelprodukte die rykste natuurlike bron van gekonjugeerde linoleïensuur is. Ongeveer 45 kg van konvensionele melk moet op 'n daaglikse basis ingeneem word om genoeg gekonjugeerde linoleïensuur te verskaf vir maksimum gesondheidsvoordele. Die doel van hierdie studie was dus om die gekonjugeerde linoleïensuur inhoud van die jogurt te verhoog tot vlakke nader aan die ADI.

Die eerste benadering was om die gekonjugeerde linoleïensuur vlakke in volroom en vetvrye jogurt natuurlik met geselekteerde suurselkulture en linoleïensuur bronne te verhoog. Drie mees algemene kommersiële jogurt suurselkulture (YC-180, YC X11 en ABT-5) is gebruik. Linoleïensuur en sonneblomolie was gebruik as bronne van linoleïensuur en was bygevoeg tot die volroom jogurt en vetvrye jogurt om 'n konsentrasie van 1 mg linoleïensuur /ml media te voorsien. Die volroom jogurt het 'n beduidend ($p < 0.001$) hoër totale gekonjugeerde linoleïensuur inhoud as die vet vrye jogurt gehad. Vetvrye jogurt met linoleïensuur het 'n effens hoër gekonjugeerde linoleïensuur inhoud as die vetvrye jogurt kontrole en vetvrye jogurt met sonneblomolie gehad. Die hoogste gekonjugeerde linoleïensuur konsentrasie in die volroom jogurt was verkry deur die gebruik van die suurselkultuur YC-X11, en die hoogste gekonjugeerde linoleïensuur konsentrasie in die vetvrye jogurt was met die suurselkultuur YC-180 verkry. Ongeveer 15 kg van hierdie natuurlik gekonjugeerde linoleïensuur verrykte jogurt moet derhalwe

daagliks ingeneem word om maksimum gesondheidsvoordele te kan verkry. Dit is nader aan die ADI as vir konvensionele jogurt, maar is nog steeds vêr van voldoende.

Die tweede deel van die studie was dus om gekonjugeerde linoleïensuur vlakke in die jogurt te verhoog deur direkte verryking met sintetiese gekonjugeerde linoleïensuur (Tonalin® 60-WDP). Die Tonalin® is bygevoeg in vier behandelingsvlakke (0 %, 1.25 %, 2 % en 5 %) en die invloed van die Tonalin® oor 'n ses-week opbergingstydperk was geëvalueer. Die totale gekonjugeerde linoleïensuur per 100 g jogurt neem toe met verhoogde Tonalin® vlakke. Die hoogste konsentrasie van gekonjugeerde linoleïensuur was verkry in die 100 g jogurt met 'n 5 % Tonalin® insluitingsvlak, wat ongeveer 2.8 g gekonjugeerde linoleïensuur oplewer. Bergingstyd het geen beduidende ($p < 0.001$) invloed op die totale gekonjugeerde linoleïensuur inhoud van die jogurt gehad nie. Dit beteken dat 'n 5 % Tonalin® insluiting 100 % van die ADI verteenwoordig. Met ander woorde, 'n verbruiker sal 100 % van die ADI vir gekonjugeerde linoleïensuur met die verbruik van slegs 100 g van die gekonjugeerde linoleïensuur verrykte jogurt kan inneem. Sensoriese evaluasie op die jogurt met die vier Tonalin® insluiting vlakke was ook gedoen. Die jogurt met die twee laagste Tonalin® insluiting vlakke (0 % en 1.25 %) het beduidend ($p < 0.001$) hoër tellings as die jogurt met die twee hoogste Tonalin® insluiting vlakke (2.5 % en 5 %) behaal. Die tellings vir die jogurt van al vier die Tonalin® insluitingsvlakke, was tussen 6 (hou effens van) en 8 (hou baie van), wat beteken dat nie een van die jogurt met enige behandelingsvlak van Tonalin® nie deur die paneel aanvaar was nie.

Daar is vasgestel dat gekonjugeerde linoleïensuur verryking van jogurt wel moontlik is. Natuurlike gekonjugeerde linoleïensuur produksie deur jogurt suurselkulture verhoog die gekonjugeerde linoleïensuur vlakke, maar gekonjugeerde linoleïensuur vlakke gelyk aan of nader aan die ADI was verkry met direkte gekonjugeerde linoleïensuur verrysking. Direkte verryking met sintetiese gekonjugeerde linoleïensuur kan dus beskou word as 'n meer realistiese benadering tot die ontwikkeling van' n nuwe funksionele suiwel voedselproduk.

Sleutelwoorde: gekonjugeerde linoleïensuur, linoleïensuur, sonneblomolie, verryking, suursel kultuur, sinteties, sensories.