Environmental factors on fatty acid composition and its impact on the assessment of marbling

Ron Tume

Food Science Australia, Cannon Hill, Qld, 4170

Phone: 07 3214 2007; Email: ron.tume@foodscience.afisc.csiro.au

Abstract. Marbling refers to the appearance of white flecks of fatty tissue between muscle fibres. The whiteness and opacity of the fat is important for visual assessment of marbling and depends on the crystallisation of the triacylglycerols within the cells. In the living animal fat is in a liquid state but with chilling the triacylglycerols undergo phase changes and become opaque. The temperature at which this occurs is largely dependent upon the melting points of the individual fatty acids. Marbling fat can comprise a diverse range of fatty acids and each has an individual melting point (e.g. palmitoleic melts at 0ºC; stearic melts at 70ºC). The visual appearance of marbling will thus depend on the melting points of the constituent fatty acids at chiller temperature. The high melting point stearic acid can vary significantly in content across groups of cattle and has a major influence on the physical properties and visual appearance of marbling fat. In this paper the effects of nutrition, seasonal and climatic variation are reviewed and linked to marbling appearance and fat hardness.

Introduction

The production of well-marbled beef is an expensive operation that requires rigorous control at each stage from cattle selection, husbandry practices, extensive feeding programs and transport of cattle to the abattoir. The overall value of each carcass will depend almost entirely on its marbling score, given that there are no problems that detract from quality issues such as dark meat colour. Therefore, in order to achieve high returns for the product, it is essential that each carcass be assessed for marbling score under conditions that are optimal for visualisation.

Marbling of meat refers to the appearance of white flecks or streaks of fatty tissue between the muscle fibres. Marbling fat is an adipose tissue, comprising fat cells (adipocytes) located in the interfascicular spaces, embedded in a connective tissue matrix and in close proximity to a rich blood capillary network. At the early stages of the development of marbling, individual fat cell diameters are generally quite small (about 40-60mm) and clusters of 10-15 cells may be required before marbling becomes visible. However, as the number of adipocytes in a location increases there is also a large increase in their size (Moody and Cassens, 1968). The appearance of a fine, evenly distributed marbling pattern is highly regarded by the Japanese market.

Marbling fat is sometimes referred to as *intramuscular* fat but marbling fat is structurally and compositionally distinct from fat or lipid present within the muscle cells (myocytes). Authentic intramuscular fat (that fat within muscle cells) largely comprises the lipid in cell membrane components (mainly phospholipids) together with that present in vesicles (mainly triacylglycerols), which can act as an energy source for muscle metabolism and contraction.

More than 90% of marbling fat cells are made up of lipid, mainly as triacylglycerols. There is a diverse range of fatty acids comprising the triacylglycerols and each has individual melting properties (e.g. palmitoleic melts at 0°C, stearic at 70°C). In the live animal or in warm meat, this lipid is in a melted state and is not visible but with carcass chilling the fat crystallises and becomes opaque and visible against the red muscle. Variations in fat composition can affect the appearance of marbling.

Importance of fatty acid composition on marbling

Lipid extracted from dissected marbling fat is essentially triacylglycerol but its fatty acid composition may vary considerably from one animal to another (Table 1). With different fatty acid compositions, the distribution of molecular

Table 1 Ranges of fatty acids (percentage distributions) present in subcutaneous and intermuscular beef fat and their melting points

Fatty acid		Range (%)	Melting point (°C)
Myristic	C14:0	2 - 4	53
Palmitic	C16:0	22 - 28	63
Palmitoleic	C16:1	1 - 12	0
Stearic	C18:0	4 - 30	70
Trans-vaccenic	C18:1,t11	1-12	45
Oleic	C18:1,c9	35 - 50	16
Linoleic	C18:2	1 - 2	-9

species of triacylglycerols will vary (Yang *et al.* 1999a; also see Fig. 1). Generally, the composition of marbling fat is similar to subcutaneous fat from the same animal but always contains a higher percentage of saturated fatty acids such as stearic acid (Table 2). Because of the difficulty in obtaining reasonable quantities of fat, most compositional studies have been performed using subcutaneous or intermuscular fat.

Palmitic acid (C16:0), a major component (about 25% of total acids) remains reasonably constant (22-26%) but stearic acid may vary from 5 to 30%, being replaced by the mono-unsaturated acids. Pure stearic acid has a melting point of 70°C whereas most of the mono-unsaturated fatty acids melt at <16°C. Thus, as the fatty acid composition changes, the overall melting properties of the fat (and thus its softness and appearance) will change markedly. Slip points (indicators of melting properties) of bovine fat were found to vary between 22.8 and 45.1°C for various groups of cattle (Smith *et al.* 1998), indicating the extent of the differences that may occur. These have been related to stearic acid content (Fig. 1). As the content of stearic acid increases from 7 to 25%, the amount of the very high melting point tri-saturated triacylglycerols increases dramatically from just 1% to 15% of all molecular species (Smith *et al.* 1998). Thus, for meat having identical fat contents, there may be large differences in the visual appearance of marbling where the melting properties of the fat differ significantly.

In addition to fatty acid composition, the position of individual fatty acids in the triacylglycerol structure will affect melting properties of a lipid. There are numerous possible permutations for positioning of individual fatty acids but, as a result of biochemical specificity (Enser 1995), in cattle, the mono-unsaturated oleic acid is preferentially located in the *sn*-2 position (Fig. 2). Where a saturated fatty acid is located at the *sn*-2 (centre) rather than either *sn*-1 or *sn*-3 (outside) position, the resulting triacylglycerol will have a lower melting point. For example, for a triacylglycerol containing palmitic, oleic and linoleic acids the melting point is –10°C when palmitic acid is located at the *sn*-2 position compared with +13°C when the central position is occupied by oleic acid (Gunstone *et al.* 1994).

Physical properties of fats, appearance, opacity and hardness are influenced by the nature of the polymorphic forms of triacylglycerols. These properties are difficult to predict, even with pure mono-fatty acid triacylglycerols but becomes even harder to predict when dealing with fat of the complexity of beef triacylglycerols.

As fat cools from the melted or liquid state (live animal at ~ 40°C) it undergoes a series of transformations and enters

Table 2. Differences in fatty acid composition (percentage distributions) of subcutaneous (SC) and dissected marbling fat from Angus and Wagyu-Angus cattle

	Angus			Wagyu-Angus		
	SC fat	Marbling fat	Signif.	SC fat	Marbling fat	Signif
14:0	2.7	2.8		3.0	3.0	
16:0	25.7	25.6		26.5	25.8	
16:1	3.5	2.7		4.5	3.2	
18:0	12.1	15.8	**	10.1	13.7	**
18:1	48.2	46.1		47.7	46.4	
18:2	1.4	1.3		1.3	1.3	
% sat	43.1	46.6	**	42.2	44.9	**
% mono	53.6	50.3	**	54.5	51.2	**
% poly	1.7	1.5		1.7	1.4	

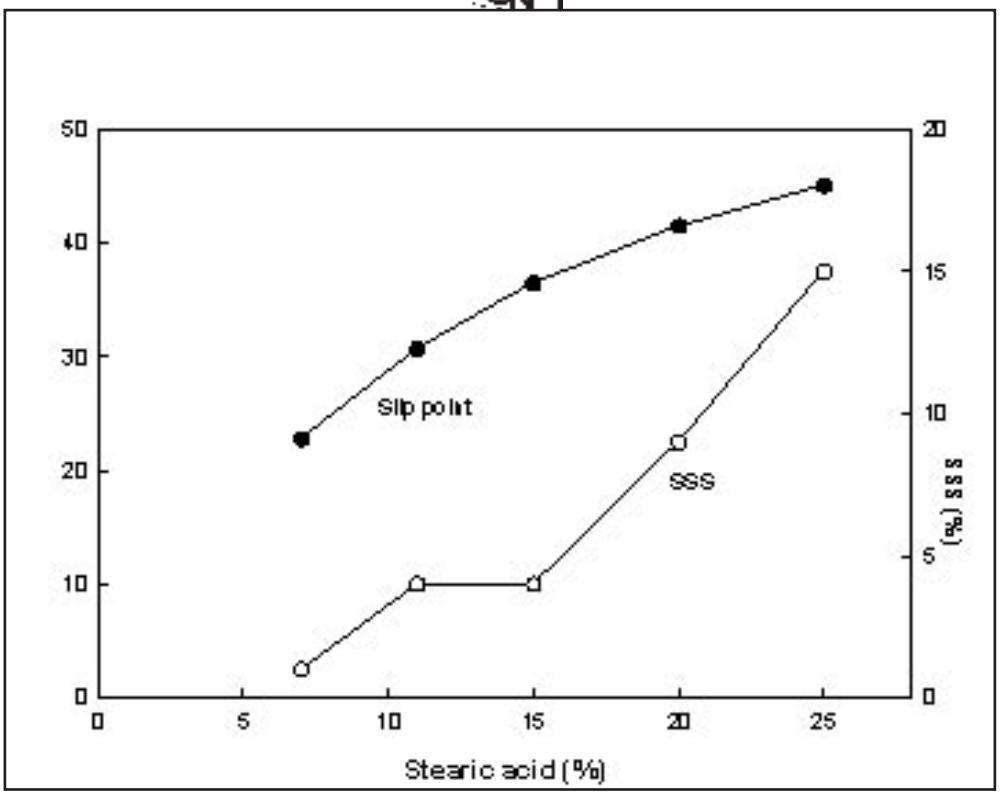


Figure 1. Effect of stearic acid content on fat composition (content of tri-saturated triacylglycerol) and slip points. SSS = tri-saturated triacylglycerol.

different polymorphic states. As a result of the large number of possible structural permutations for triacylglycerols, broad, ill-defined changes are observed. Despite this, fat will set initially in a triclinic crystalline form (μ) and then, with time, as either of several forms of monoclinic structures (b^1 and b), with the b form being most stable. Because bovine triacylglycerols predominantly have an unsaturated fatty acid in the *sn*-2 position, they tend to form b^1 structures although some will form b structures with increased duration of storage. As these different structures affect the reflection of light, the physical appearance of the fat is affected, appearing more opaque.

Insight into the thermal properties of subcutaneous fat, particularly as it relates to events occurring during carcass chilling, has been gained by differential scanning calorimetry (DSC). Studies on bovine subcutaneous fat revealed that major phase transitions occurred at about 8 –15°C and also at about 35 – 40°C (Yang *et al.* 1999). Depending on the level of unsaturation of the fat, the enthalpy associated with each transition was markedly different, with the more unsaturated samples showing higher enthalpy changes in the lower temperature ranges. Thus, at chiller temperatures of around 10°C, the appearance of the fat (or opaqueness) will depend on the proportion of the fat that has undergone the phase transition. In meat where the marbling fat is more unsaturated, compared with others and where there is incomplete phase transition, the fat will be partly translucent (less white) and will not appear boldly against the red muscle background. Under these conditions, it would be expected that chiller assessment of marbling would result in a lower marbling score than that where the fat is more saturated.

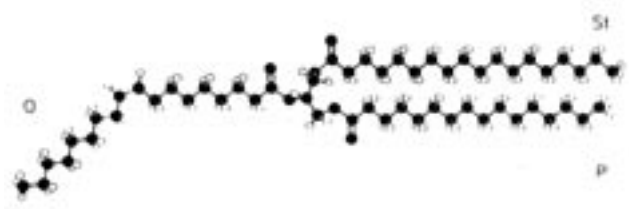
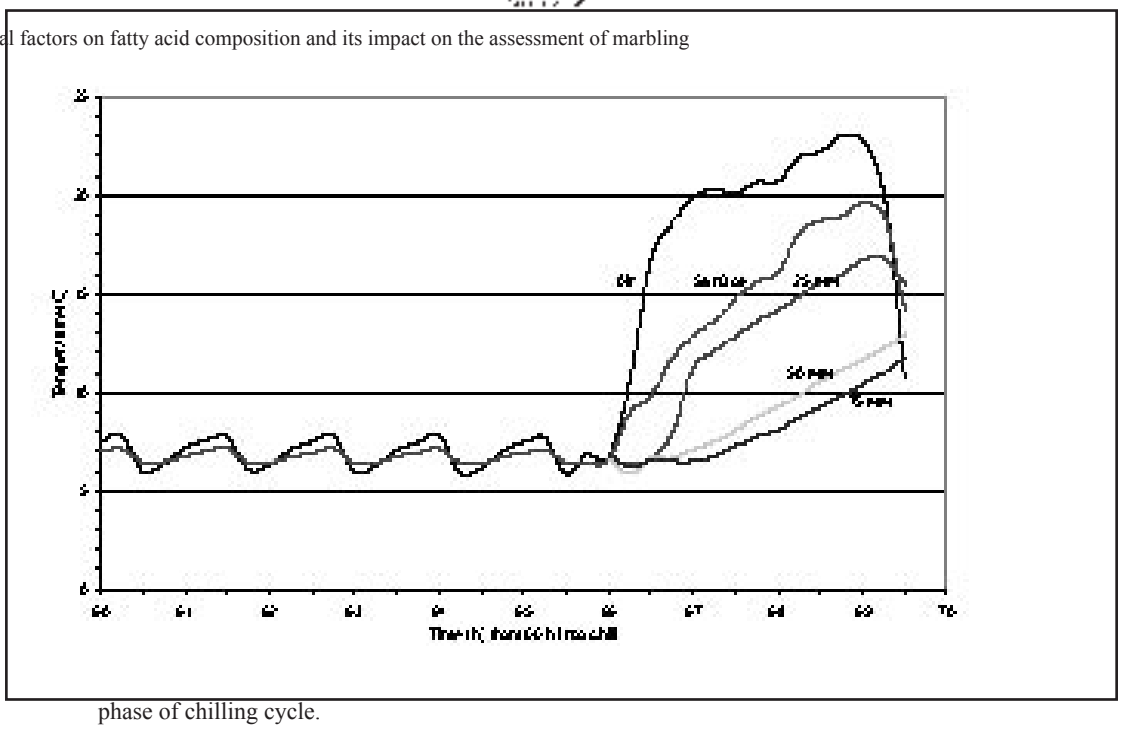


Figure 2. Structure of a tri-acylglycerol containing stearic (St), oleic (O) and palmitic (P) acids at the *sn*-1, *sn*-2 and *sn*-3 positions respectively. Diagram from Loders Croklaan in Beckett (2000).

Temperature and time effects

From the above it can be seen how fatty acid and triacylglycerol structures are affected by temperature. The importance of temperature on visual marbling score is illustrated by the work of Pethick *et al.* (1997) where 107 carcasses were initially assessed under commercial conditions (day after slaughter, carcass temperature 11-12°C) and then re-assessed 24 hours later at 5°C. At initial assessment, only 41 carcasses achieved a marbling score of >2 whereas after a further 24 hours with chilling to 5°C, 51 had scores of >2.

It is, therefore, imperative that marbling assessment is performed on carcasses at as low a temperature as possible if marbling scores are to be properly compared. Carcass chilling



phase of chilling cycle.

procedures are usually a compromise between food safety requirements and the boning problems associated with hard carcass fat. It is common practice to chill to a loin temperature of about 5°C at about 20 hours and then to commence carcass rewarming for about 3 hours to minimise the hard fat problem. For longer chilling cycles, as required over a weekend carcasses may be chilled to 5°C and then the temperature held at 10 for more than 24 hours or alternatively subjected to a 3-hour reheating cycle immediately prior to boning. In one study (Fig. 3) it was found that warming carcasses (about 330kg) with air at temperatures of about 20°C resulted in the surface temperatures rising to about 13°C at one hour and to more than 10°C at a loin muscle depth of 25mm. Three hours of warming resulted in the meat at 50mm depth reaching 11 to 12°C. Chiller assessment of marbling should be performed prior to any rewarming cycle.

What environmental factors affect the stearic acid content of marbling fat?

Rather than consider all fatty acids, we believe, for the reasons given above, that the variation in content of stearic acid in fat is the major determinant of the differences in its physical properties. Therefore, in the following section, the effect of environmental factors on stearic acid content is discussed. Given that very few studies have been performed on dissected marbling fat, this work considers those effects observed for subcutaneous or intermuscular fats.

Nutrition

Although usually low in total fat content, pasture grasses and various grains contain high proportions of poly-unsaturated fatty acids, in particular, linolenic and linoleic acids, respectively. Most of these unsaturated acids are hydrogenated by rumen microflora and consequently, the fatty acid composition of the digesta that reaches the small intestine is very different from that of the diet. Although stearic and *trans*-vaccenic acids are the major fatty acids available for absorption (>60 to 70%), the composition of adipose tissue fat suggests that dietary lipids are significantly metabolised and/or that *de novo* synthesis of fatty acids is a major pathway for the accumulation of lipid in fat cells. Importantly, a number of tissues including intestinal mucosal, liver and fat cells each contain an enzyme system, D^9-desaturase that converts saturated to mono-unsaturated fatty acids (eg. stearic into oleic acid). There is also some evidence that this enzyme is capable of desaturating *trans*-vaccenic to conjugated linoleic acid in lactating dairy cows (Griinari *et al.* 2000).

For the synthetic pathway, where fatty acids are built up from 2-carbon units to C16 saturated fatty acids, elongase and desaturase are important for the production of the longer, mono-unsaturated acids such as oleic (C18:1,c9). Thus D^9-desaturase plays an extremely important role in determining the overall saturation of fatty tissues.

Many studies have compared the effects of pasture versus grain feeding on the fatty acid composition of bovine adipose tissues but such studies are difficult to adequately control (different weight, age, fatness etc) and have lead to variable findings in relation to overall saturation. Some have reported that grain feeding leads to decrease in the percentage of stearic acid (Rumsey *et al.* 1972; Marmer *et al.* 1982), usually being replaced by oleic acid. An investigation with tropically adapted cattle Kelly *et al.* (2001) found that finishing did not affect the content of stearic acid in steers but pasture-fed heifers had significantly less than those grain-fed. In other studies, we have reported (Yang *et al.* 1999a) that grain feeding can result in high contents of stearic acid (18.7 ± 0.45%) but this is clearly not always the case as very low contents of stearic acid (10.4 ± 2.6%, Siebert *et al.* 1996 and 10.5 ± 0.9%, Smith *et al.* 1998) have been obtained. In the latter cases, cattle were fed on a barley-based diet (up to 80%) or corn for 300 days

in southern Australia regions as opposed to cattle being fed commercial rations for more than 300 days in northern NSW. However, in a large study of 764 grain-fed cattle covering 7 breeds, Malau-Aduli *et al.* (2000) observed that stearic acid contents ranged from 12 to 23 % and had a mean value of 13.5%.

Table 3. D^9-desaturase activity and stearic acid contents of subcutaneous fat in cattle on different diets (see text for details).

	D^9-desaturase activity (nmol/mg protein/min)	stearic acid (%)
Control	0.83 ± 0.07	13.1 ± 1.00
Protected cottonseed oil	0.40 ± 0.04	18.5 ± 0.54

Because of the diversity of feed types, both in pasture and grain, coupled with differences in animal growth rate, stage of development and perhaps climatic conditions, it would seem unwise to categorically state that one finishing system or another leads to a more saturated fat or a certain fat composition. More importantly, feed ingredients need to be considered for any possible effect they may have individually, or in combination. For instance, there is considerable anecdotal evidence that feeding whole cottonseed can result in hard carcass fat. Fat from these carcasses, compared with others, has a higher content of stearic acid with lower oleic and palmitoleic acids, suggesting that there has been an inhibition of D^9-desaturase activity within the tissues.

Cyclopropenoic fatty acids

The oil of whole cottonseeds contains two cyclopropenoic fatty acids (sterculic and malvalic), which are known potent inhibitors of D^9-desaturase activities and have been shown to increase the degree of saturation (increase stearic acid content) in a number of non-ruminant species (Carter and Frampton 1964; Nixon et al. 1974). While the effect of these dietary unsaturated acids in cattle is not as great as in non-ruminants (as a result of some hydrogenation in the rumen), a portion are absorbed intact, leading to an inhibition of D^9-desaturase activities (Yang et al. 1999b). Although in other work, from the US, there was no effect (Huerta-Leidenz et al. 1991; Page et al. 1997). However, experimental feeding trials with varying amounts of whole cottonseed need to be done to quantitatively determine its impact on fat composition.

The total cyclopropenoic fatty acid content of seeds from 25 varieties of cotton grown in northern and southern locations of the cotton-growing districts over several seasons was found to be quite constant, with means varying from about 0.4% to about 0.7% (Tume 1999), similar to values reported by Wood (1986) from the US. In a trial with cattle fed pasture or grain containing 5% whole cottonseed for 100, 200 or 300 days we found that D^9-desaturase activities of subcutaneous fat were significantly lower (1.48 versus 0.82nmol/mg protein/min, $P<0.01$) for the grain-fed groups, but no direct comparison was possible for grain without added cottonseed.

In other work, we were able to directly compare fat from cattle that had been fed essentially the same grain ration for 140 days but one group was supplemented with 5% rumen-protected cottonseed oil for the last 80 days. The following tabulation shows the mean (± standard errors) for stearic acid contents and D^9-desaturase activities of subcutaneous fat of the two groups (Tume *et al.* 1997):

Clearly, feeding a ration in which cyclopropenoic fatty acids are protected from rumen degradation leads to inhibition of desaturase activity and, thus, an increase in stearic acid content. It is, therefore, likely that when feeding whole cottonseed, some cyclopropenoic acids will escape hydrogenation and result in an increase in saturation of fat within the animal.

Trans-fatty acids. Although this paper is primarily involved with the effects of stearic acid on visual appearance of marbling, *trans*-vaccenic acid (C18:1, t11) should be considered because of its relatively high melting point. Unlike oleic acid (C18:1, c9) which melts at about 16°C, the trans isomer has a melting point of 45°C. Usually, the content of the trans-isomer in subcutaneous or intermuscular fat is only 2 to 3% but, for individual cattle, can reach 12% of total fatty acids, thus, significantly affecting overall hardness (and opacity) of the fat. From the CRC I trial we found that the content of *trans*-fatty acids was not only higher in grain-fed compared with pasture-fed cattle, but was significantly higher for cattle finished in northern, compared with southern, locations (Kelly 1999). Dietary treatments did differ between locations and it is likely that the high contents of *trans*-fatty acids resulted from inclusions of plant oils in the diets (O'Kelly and Spiers 1993).

Seasonal and climatic variation

Seasonal and climatic effects have been reported for fatty acid composition of ruminants (Marchello *et al.* 1967; Leat 1975; Perry *et al.* 1998). Temperature and rainfall are likely to affect fatty acid composition in several ways. Firstly, there may be differences in feeds (or feed quality) from one season to another, although that is likely to be more of an issue for pasture-fed rather than grain-fed cattle. Secondly, changes in fat composition result from established climatic differences (colder versus warmer regions). However, any such differences resulting from direct temperature effects are most likely to occur only in subcutaneous fat, because of the temperature homeostasis of the deeper tissues. Fatty acid composition changes to maintain constant lipid fluidity within the fat cells to allow for normal metabolic function. This regulation is likely to be primarily achieved by D^9-desaturase activities (Kouba *et al.* 1999) with activity being greater in cooler tissues.

Attempts to compare any such environmental effects in cattle have generally proved difficult, as feed type will always vary. Whilst there are many examples of cattle from more southerly regions having lower stearic acid contents in adipose tissues compared with northern regions (Siebert *et al.* 1996; Yang *et al.* 1999), diet cannot be eliminated.

Analysis of fatty acid data from CRC I cattle (n=1052) from temperate regions revealed significant seasonal variations in

stearic acid contents of subcutaneous fat in pasture-fed but not in grain-fed cattle (Kelly 1999). Using parameters of a fitted sine curve to the fatty acid data as a function of Julian day over about 2 years, it was found that even though significant ($P<0.001$) for pasture-fed cattle, the predicted amplitude was only about 1.4%. For grain-fed cattle variation was not significant ($P=0.771$) and the predicted amplitude was only 0.15%. Thus for grain-fed cattle essentially no variation in stearic acid would be expected for the more deeply located marbling fat.

Summary

Not only are Japanese markets demanding product with increased marbling but also meat with softer fat. Whilst it is in this direction of producing softer fat that the industry appears to be moving, and one that clearly has benefits for carcass boners, it does have negative implications for the extent of marbling as measured in Australia. As feeding systems to enhance the marbling fat content of meat have often lead to a reduction in the stearic acid content of fat, marbling assessment should be performed on loins that have been very well chilled and not rewarmed.

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