Foaming behaviour of organic and regular milk

bioKennis
voor biologische agroketens

Jan Pijnenburg
Guido Sala
Hein van Valenberg
Marcel B.J. Meinders

WAGENINGEN UR
For quality of life

Colofon

In Nederland vindt het meeste onderzoek voor biologische landbouw en voeding plaats in het, voornamelijk door het ministerie van EL&I gefinancierde, thema Biologische Landbouw (van het Beleidsondersteunende Onderzoek). Aansturing hiervan gebeurt door Bioconnect, het kennisnetwerk voor de Biologische Landbouw en Voeding in Nederland (www.bioconnect.nl). Hoofduitvoerders van het onderzoek zijn de instituten van Wageningen UR en het Louis Bolk Instituut. Dit rapport is binnen deze context tot stand gekomen.

De resultaten van de verschillende kennisprojecten vindt u op de website www.biokennis.nl. Voor vragen en/of opmerkingen over het onderzoek aan biologische landbouw en voeding kunt u mailen naar: info@biokennis.nl. Heeft u suggesties voor onderzoek dan kunt u ook terecht bij de loketten van Bioconnect op www.bioconnect.nl of een mail sturen naar info@bioconnect.nl.

Foaming behaviour of organic and regular milk

Authors: Jan Pijnenburg, Guido Sala, Hein van Valenberg,
and Marcel B.J. Meinders
Affiliation: Wageningen University and Research Centre, Wageningen,
The Netherlands
Project: BO-12.10-004.01-016
Schuimbaarheid van Biologische Melk
Date: July 2012.

Abstract

Organic milk is used more and more by consumers to froth milk that is used e.g. for the preparation of a cappuccino. Frequently, organic milk turns out not to foam properly. Here we report on a study to find the main cause of this bad foamability of organic milk. The focus of the research was to get insight in the foaming behavior of a specific brand, which we will indicate as A. We tested the foamability and stability of different commercial milk, both organic and regular, as well as skimmed, semi-skimmed, and full fat. The foamability of the different milk varieties appeared to be about equal. However, differences were observed for the foam stability. No clear relation could be observed between foam stability and fat content (skimmed vs. semi-skimmed vs. full-fat) and/or type (regular vs. organic). However, foams made from A showed the lowest stability of all tested milk varieties. The reason for this is not yet clear, but the bad foam stability of this milk might be probably due to a higher free fatty acid content. Considering the focus of this project, we did not investigate the differences between various milk varieties in more detail, but focused on the foaming properties of milk A. Therefore, we measured the foamability and foam stability of semi-skimmed and whole milk A purchased in the local stores of Wageningen, the Netherlands, in the period from September 2011 till January 2012. The surface tension, protein content, dissolved casein content, fat content, average fat globule size and free fatty acid content and composition were determined and compared to the foaming properties.

Only one whole and two semi-skimmed organic milk samples were found that showed a lower foam stability than average. This is in line with the low number of complaints in this period. No relation in foaming properties and difference in season (meadow/summer versus stable/winter period) could be observed.

No correlation was found between surface tension and foaming properties. Also no correlation was found between the protein content and the foaming properties. For dissolved casein only a weak correlation with foamability was observed for whole milk and not for semi-skimmed milk. The reason is not clear yet and needs further investigation.

No correlation was found between the fat content and foaming properties. This might be due to the fact that the frothing took place at 56°C. At this temperature there are no fat crystals present in the fat globule that could pierce the fat globule membrane so that liquid fat can be released and cause coalescence of air bubbles.

A weak correlation was found between the fat globule diameter and foamability for semi skimmed milk. Very weak correlations were found between fat globule size and both foamability of whole milk and foam stability of semi skimmed milk. No correlation was found between the foam stability and fat globule size for whole milk A.

It is expected that foaming properties are negatively influenced by free fatty acids (FFA). This was supported by our experiments where we added small amount of linoleic acid (C18) to various milk varieties. However, we also determined FFA content and composition of different milks. In these experiments, no correlation was observed between free fatty acids content and foamability. The reason for this is no clear and also needs further investigation.

Concluding, no clear relation between foaming properties of organic semi-skimmed and whole milk A and determined compositional parameters could yet be observed. Therefore, although there are indications that the amount of free fatty acids are negatively related to foaming properties of milk, no clear cause for the occasionally bad foaming properties of organic milk could yet be identified. One of the main reasons for this outcome is that only very few milk samples were found with significant worse foaming properties. Another reason might be that the studied samples were commercial bulk milk of which the history concerning technological and heat-treatments was unknown. For a better mechanistic understanding of the role of milk composition on foaming properties, both the temperature and process history should be known or controlled.

1. Contents

1. Introduction

More and more consumers use organic milk to prepare milk foams, e.g. as topping on a cappuccino. Frequently organic milk does not foam properly. The reason for this is unclear. The organic sector would like to solve this problem of bad foamability of organic milk because it hinders growth of the organic dairy market.

From preliminary research it seems likely that the occasionally bad foamability of organic milk is not related to the production process and homogenization pressure. Furthermore, it seems that the bad foaming properties of organic milk are observed more frequently when the cows are inside during the stable-period. Questions that the organic sector would like to have answered include: what is the main cause of the foamability problem of organic milk, why is there such a large variability in the foamability of organic milk, and how can the foamability problem of organic milk be solved? Here we report on a study to find answers to these questions. We focuses on the foaming behaviour of a specific brand which we will call A.

1.1 Milk foam

A foam is a dispersion of gas bubbles in a liquid. A foam has a large interfacial area. The making and maintaining of this large interfacial area costs energy. Because a system tries to minimize its total energy, foams suffer from deterioration. Three phenomena can be identified: i) coalescence, where two bubbles (of which one may be the environment) merge due to rupture of their separating films, ii) creaming or drainage, where the liquid drains to the bottom and the air bubbles cream to the top due to gravity and their differences in density, and iii) disproportionation, where gas diffuses from small bubbles to large bubbles (or the environment) due to differences in the so-called Laplace pressures of the bubbles.

In general one distinguishes the formation and the (long term) stability of a foam or bubble. It is generally believed that the formation of foam is directly related to the adsorption properties of the surfactants in the solution, while the stability is related to the rheological properties of the interface. For good foaming properties, surfactants should adsorb fast enough to the air/water interface in order to minimize the interfacial energy (Bos *et al.* 2001) and to prevent the formed gas bubbles from collapsing and coalescing (Kinsella 1981). Furthermore, the surfactants should form strong, elastic interfaces that minimize coalescence and disproportionation (Dickinson et al. 1989). These properties strongly depend of the components present.

Milk contains various surfactants that can influence the foaming behaviour both positively and negatively. Milk contains low molecular and high molecular weight surfactants. Low molecular weight surfactants (LMWS), like free fatty acids

(FFA), monoglycerides, and diglycerides are mostly found in the fat phase. Fat is present in fat globules that are surrounded by a fat globule membrane, which consists of phospholipids. The serum phase also contains a small quantity of peptide fragments. High molecular weight surfactants (HMWS) in milk are the whey proteins, like ß-lactoglobulin and α-lactalbumin, and the casein fractions, like α_{s1}-, α_{s2}-, ß- and k-caseins (Dickinson 1989, Brooker *et al.* 1986).

Despite the amount of research performed on the formation and stability of foams, there are still no generic rules that describe the foaming properties of multicomponent foams that consist of various ingredients and different types of surfactants like milk.

For example, although FFA adsorb fast and lowers interfacial tension quickly, their presence in milk has a negative effect on the foaming properties. This is due to the fact that FFA can displace proteins from the air/water interface, thereby destabilizing the interface (Kamath S. 2008).

Furthermore, the different proteins present in the milk have different chemical and physical properties, like adsorption rate, adsorption energy, surface rheology. Therefore, the foaming properties of the milk will depend on the concentrations of the different surface active components (Dickinson, 2010, Zhang, 2003, Prins, 1999, Graham & Phillips, 1975, Cornec *et al.* 1996, Mackie A.R., 2000).

Fat content has also a strong effect on the foaming properties of milk. Even small volumes of fat may negatively influence foamability when a foam is formed at a temperature of 10-40°C (Huppertz, 2010). In this temperature range a part of the fat is liquid and a part is crystalized. The fat crystals can pierce the fat globule membrane resulting in liquid fat being released and spreading along the air/water interface. This leads to bubble coalescence. Therefore, foaming should preferably be performed at temperatures above 40°C. Another way to prevent rupture of the fat globule membrane by fat crystals is homogenization of the milk. Homogenization reduces the size of the fat globule and fat crystals. After homogenization the newly formed fat globule membrane consist of casein micelles and more difficult to be pierced by the fat crystals (Huppertz, 2010).

Also the competitive adsorption of both MLWS and proteins can have a destabilizing effect. LMWS mainly stabilize foams via the Gibbs-Marangoni mechanism because they form a fluid-like or viscous interface. Proteins mainly stabilize foams by forming strong viscoelastic, immobile adsorbed layers (Chen, 1995). When both LMWS and proteins adsorb, gradients in surface tension might cause film thinning of the protein film, which will finally result in film rupture (Wilde, 2004). Furthermore, also the drainage rate will increase with increasing amounts of adsorbed LWMS with respect to proteins, because in that case the interface becomes more fluid-like.

Milk is a natural product. Therefore the concentration of the various components present in milk that effects foaming properties will fluctuate with e.g. season, breed, stage of lactation, and feed (Holden *et al,* 1966). Furthermore, due to the presence of enzymes and micro-organisms in milk, the composition and thus foaming properties might also change during storage.

1.2 Compositional difference between organic and regular milk

Generally, cows that deliver regular milk graze more inside and are fed with higher amounts of concentrate than cows that deliver organic milk (Gustafson, 2003). This difference in feed will lead to a difference in free fatty acid composition between organic and regular milk (Molkentin, 2007, Couvreur et al. 2006, Ferlay et al. 2006, Markus Schroder *et al. 2010*). In table 1.1 the largest differences in composition between organic and regular milk on the bases of difference in diet is shown.

Table 1. Dry mass, fat content and content in 4 different fatty acids of milk from cows fed either organic of regular feed

	Organic	**Regular**
Dry mass (%)	12.5 ± 1.0	13.9 ± 0.6
Fat in dry mass (%)	26.9 ± 6.6	32.3 ± 3.5
Fat in whole milk (%)	3.4 ± 1.0	4.5 ± 0.6
α-Linolenic acid 18:3n-3 (mg/100 g fat)	906 ± 86	403 ± 29
Phytanic acid (mg/100 g fat)	314 ± 36	146 ± 16
eicosapentoic acid 20:5n-3 (mg/100 g fat)	109 ± 9	45.4 ± 4.3
Pristanic acid (mg/100 g fat)	36.3 ± 4.1	25.9 ± 3.4

Organic milk usually contains more long chain fatty acids than regular milk. Furthermore, it has been shown that linoleic acid rich milk contains more long chain fatty acids on position sn-1 and sn-3 of the triglycerides (Nilsson-Ehle 1973). The lipoprotein lipase is present in all bovine milk at a content between 1-2 mg/L. It hydrolyses FFA from triglycerides with a preference for the FFA at the sn-1 and sn-3. Therefore, assuming equal lipase activity, during storage the FFA concentration in linoleic acid rich milk will increase faster than in regular milk. Consequently, the foaming properties of linoleic acid rich milk, i.e. of organic milk, will decrease more than that of regular milk,

1.3 Aim and objective

The aim of this research was to find the main cause of the occasional bad foaming behaviour of organic milk. Therefore, we followed the foamability and foam stability of different commercial milks, both organic and regular, as well as skimmed, semi-skimmed and full fat, during the period between August 2011 till January 2012. Parameters like surface tension, dissolved casein content, protein content, fat content, size of the fat globule, and FFA content were determined.

2. Materials and methods

2.1 Materials

2.1.1 Milk samples

In the period September 2011 till February 2012, two to three times a week milk samples of various types and brands (1L packages) were bought in the local stores of Wageningen, the Netherlands.

2.2 Methods

For all studied milks, foamability and foam stability were determined. After purchasing of the milk, part of a certain selection was analysed the same day for foamability, foam stability, fat globule size, total fat content, total protein content, and surface tension. Another part (250 ml) was stored for two days in the fridge at 7°C for the analysis of dissolved casein and NPN. The two days of storage at 7°C were meant to minimize possible difference in temperature history. Also another part (50 ml) of the milk was stored in the freezer at -32°C to be analysed for FFA content by gas chromatography.

Furthermore, foam stability tests of milk samples to which linoleic acid was added were performed to investigate the effect of FFA on the foam stability.

2.2.1 Foaming properties

Milk samples of 100 ml (103 g) were frothed using the Nespresso Aerocinno 3 (Nestlé Nespresso, USA), an automated system for the frothing of milk. The frothing time was 1 minute and 10 seconds. During frothing the milk was automatically heated to about 56°C. After frothing the milk, the foam was poured into a graduated cylinder and the total volume as well as the volume of the drained liquid was measured as a function of time. The overrun is defined as:

$$\varphi = \frac{V_{tot-}V_{ld}}{V_l} = \frac{V_{foam}}{V_l} \qquad (1)$$

where V_{tot} is the total (foam+liquid) volume, V_{ld} is the liquid drained at the bottom of the tube, V_l=100 ml is the initial liquid volume, and V_{foam} is the foam volume.

The overrun as a function of time showed a characteristic shape and was fitted using the following equation

$$\varphi(t) = \frac{\varphi_1}{t_1+t} + \varphi_p\left(1 - \frac{1}{1+\exp((t_c-t)/t_2)}\right) \qquad (2)$$

The first part describes the drainage and the second part the collapse of the foam due to coalescence and disproportionation. Fitting parameters are φ_1, t_1, Φ_p, t_c, and t_2. The overrun at t=0 ($\varphi(0)$) and the plateau value φ_p are used as a measure of the foamability, and t_c, corresponding to the time the foam collapses, is used as a measure for the foam stability; t_1 corresponds to the drainage time.

For some of the experiments where FFA was added to the milk, the overrun could not be modelled with equation 2. In that case also the foam half-life $t_{1/2}$, corresponding to the time after which the foam height has decreased to half its initial value $V_{tot,0}$, was taken as a measure for the foam stability.

$$t_{1/2} = t(V_{foam} = 0.5 V_{foam,0}) \qquad (3)$$

2.2.2 Surface tension

The surface tension was measured using a Wilhelmy plate and a force transducer (Wagezelle, Typ: Q11, Germany). A glass Wilhelmy plate (20x26mm) (Menzel glaser, Germany) was used. Two weights of known force (41.5 mN and 71 mN) were used to calibrate the surface tension meter. The glassware used for the determination of the surface tension was cleaned and thoroughly rinsed with tap water.

For the measurement, milk samples of 100 ml were placed in a pyrex beaker glass (85 mm diameter) at room temperature (20°C). After 10 minutes, when fluctuations were reduced, the data recorder was started for 1 minute.

2.2.3 Total protein and fat content

A Milko-scan (Milko-Scan 134A/B, N. Foss Electric, Denmark) was used to determine the total fat and protein content. All milk samples were preheated to 40°C in a water bath. Each sample was measured for 5 times in a row. The data of the last two measurements were noted.

2.2.4 Dissolved casein and non-protein nitrogen (NPN)

For the determination of the protein content of the samples, DUMAS (Flashea 1112 series, Interscience, Breda, the Netherlands) was used. The settings of DUMAS was 900°C for the left furnace, 680°C for the right furnace and 50°C for the oven. The carrier gas was helium with a flow of 140ml/min; the reference gas was set at 100ml/min.

The milk samples were prepared as follows. Milk (250 ml) was centrifuged at 25.000 rpm at 4°C for 30 minutes. Fat was separated from the milk and the skimmed milk was pipetted in centrifuge tubes (20 ml). The skimmed milk was subsequently ultracentrifuged at 40.000 rpm at 4°C for 75 minutes to separate the casein micelles from the sample. After ultracentrifugation the supernatant of two out of four tubes was acidified with hydrochloric acid to pH 4.6 to precipitate the dissolved casein. The acidified samples were ultra-centrifuged again at 40.000 rpm at 4°C for 30 minutes to separate the dissolved casein from the sample. The protein content of both the non-acidified and the acidified supernatant was determined in order to calculate the content in dissolved casein of the milk. The supernatant (± 10 ml) was pipetted in a container tube. 200 µl was pipetted in a tin cup; the weight of the sample in the tin cup was noted. The cup was dried in a stove for 24 hours at 40°C. The tin cup was closed and put in the sample tray for Dumas analysis.

The dissolved casein content was assumed to be equal to the difference in protein content between the acidified and non-acidified supernatants.

2.2.5 Fat globule size distribution

The Mastersizer (Malvern instruments,UK) was used for the determination of the size distribution of the fat globules. Particle refractive index was set at 1.480 and the particle absorption index used was 0.01. All samples were analysed in duplicate.

2.2.6 Free fatty acids (FFA) content and composition

The following method was used to isolate the free fatty acids from the milk. 10.0 ml milk was mixed with 10 ml ethanol, 1 ml sulphuric acid (2.5 mol/L) and 1 ml internal standard solution. 15 ml ether/heptane (1:1 v/v) was added to the sample and shaken. The upper solvent layer was transferred to a 100 ml conical flask containing 1 gr anhydrous sodium sulphate. 10 ml ether/heptane (1:1 v/v) was added to the sample, which was subsequently centrifuged at 2500 rpm for two minutes at room temperature. The upper solvent layer was transferred to a 100 ml conical flask. 10 ml ether/heptane (1:1 v/v) was added to the sample. The centrifugation procedure was repeated. The upper solvent layer was transferred to a 100 ml conical flask. Aminopropyl columns were conditioned with 10 ml heptane. The lipid extract was applied to the column. Neutral lipids were eluted from the column with chloroform/2-propanol (2:1 v/v). Free fatty acids were eluted with diethyl ether containing 2% formic acid. 50 µg C9:0 injection standard was added to the solution. A sample of 0.5 µl was taken for gas chromatography

Gas chromatography was performed on an Focus gas chromatograph fitted with an 3380 A Integrator Hewlett Packard (Interscience, Breda, the Netherlands). The column stabilwax DA 30m.*0.32 mm 1µm (Interscience, Breda, the Netherlands) was used. It was conditioned for 24 h at 240°C before use. The temperature profile of the column was programmed from 40°C with a 1 min hold and temperature increase rate of 10°C/min to 250°C. The ionization detector of the gas chromatograph was supplied with 25 ml hydrogen and 240 ml air/min. 5 kPa pressure and 10 ml/min splitting volume were used. The carrier gas flow was 10 ml nitrogen/min.

3. Results and discussion

3.1 Foaming behaviour various milk varieties

In order to get insights in the foamability and foam stability of the organic milk A, we first investigated the foaming properties of various milk varieties as references. Results are depicted in figure 2. It shows the averaged foam overrun of three triplicates as a function of time (symbols) and the fit using equation 2, for different types of regular and organic milk.

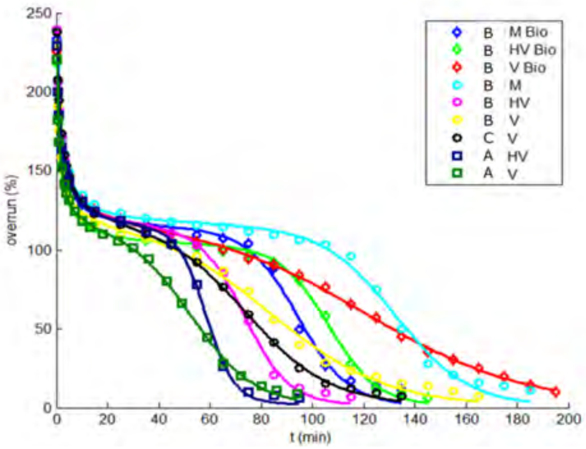

Figure 2. Measured overrun as function of time (symbols) of different types of regular and organic milk as well as the best fit using equation 2 (continuous lines). A,B, C indicate different brands; M= skimmed milk, HV= semi skimmed milk, V=whole milk; Bio=organic milk, rest is regular.

Clearly, three regimes can be identified. First, a region up to about 10 minutes where the foam volume decreases fast due to drainage. Than a region where the milk foam is more or less stable. Here the liquid volume fraction of the foam gets approximately smaller than that of the random close packing. The shape of the bubbles becomes more hexagonal and drainage is slowed down due to the increased capillary pressure in the Plateau borders. In the third regime the foam collapses due to coalescence and collapse of the air bubbles because film lamellae are drained so much that they become unstable. It is seen that these regimes can be very well modelled using equation 2.

Parameters for foamability and foam are shown in figure 3. It can be observed that the foamability was about equal for all foams, but that the foam stability showed significant differences between the different milk varieties. No clear relation could be observed between foam stability and fat content (skimmed vs. semi-skimmed vs. full-fat) and/or type (regular vs. organic). Foams made from A milk, the focus of this study, showed the lowest stability of all tested milk varieties. The reason for this is not yet clear.

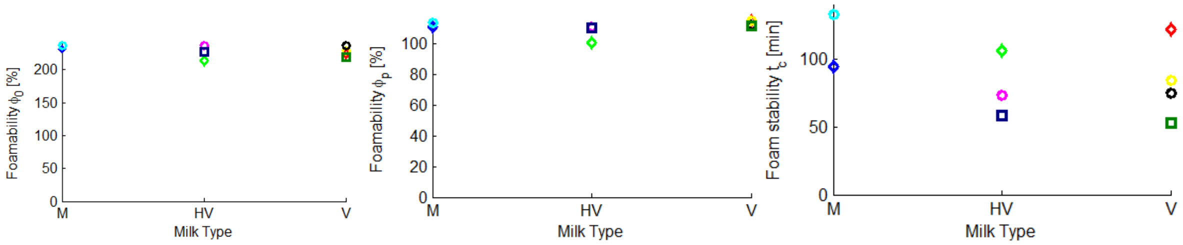

Figure 3. Foamability φ_0 (left), φ_p (middle) and foam stability t_c (right) for the different milk foams (see figure 2). The milks are grouped according to their fat content, skimmed (M), semi-skimmed (HV) and full fat (V).

3.2 Foam stability of organic milk over time

Figure 4 shows the measured overrun of foam made with various semi-skimmed and whole milk samples of brand A as a function of time. The different samples were taken at different dates in the period from September 2011 till January 2012. The figure also shows the best fits. The values of the fitted plateau border φ_p, corresponding to the foam stability, and the fitted characteristic time that the foam collapses, t_c, are shown in figure 5, grouped for the semi-skimmed and whole milk.

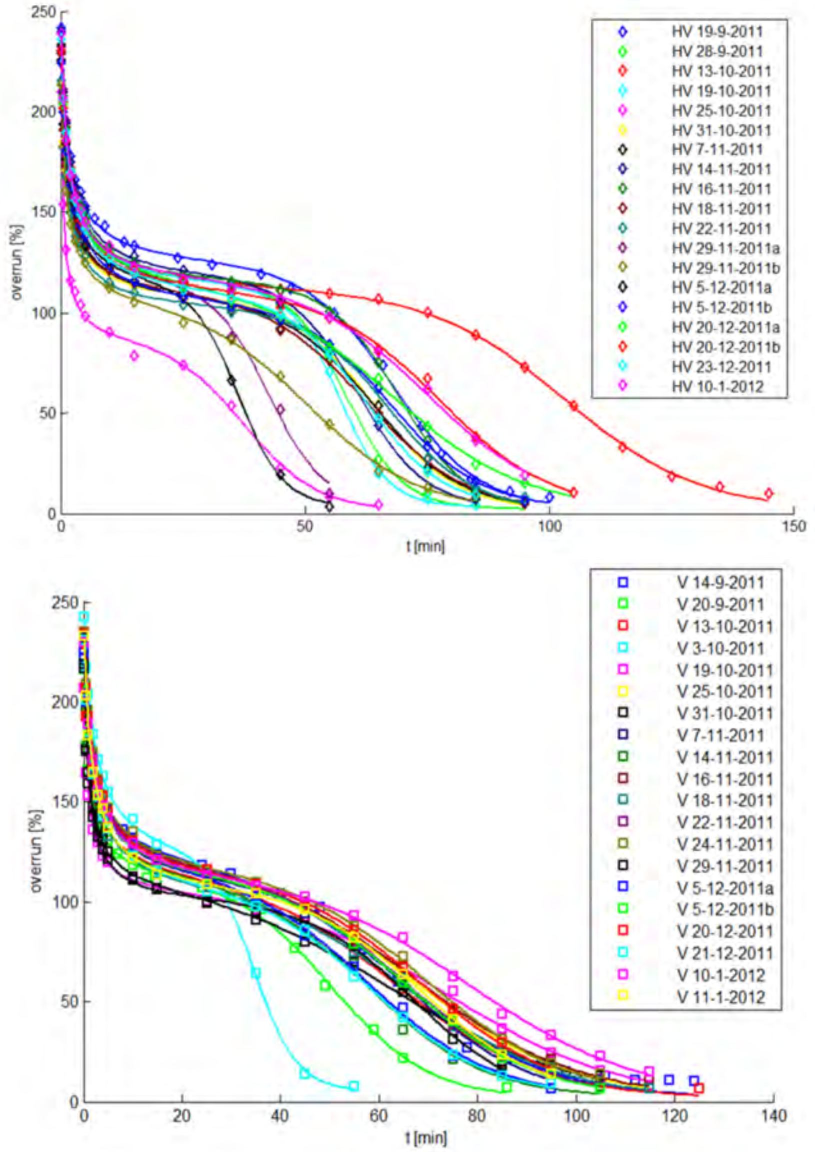

Figure 4. Measured overrun (symbols) of different whole (bottom) and semi-skimmed milk (top) samples of brand A as a function of time, as well as the best fits using equation 2 (continuous lines). The samples were taken at different dates, as indicated. HV = semi-skimmed milk, V = whole milk.

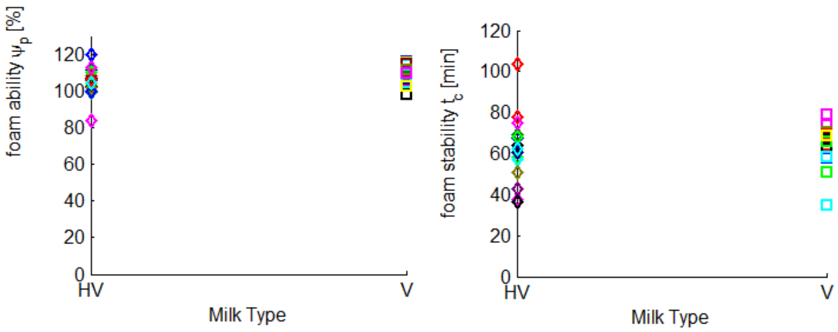

Figure 5. Fitted foam ability, plateau value φ_p, (left) and foam stability, t_c, (right) of the semi-skimmed (HV) and whole (V) milk of brand A (see figure 4).

The whole milk A from 3-10-2011 showed a significant lower stability t_c=35 min. The two most stable milk foams were prepared from the samples taken at 10-01-12 and 20-12-11. It was expected that the stability of the whole milk would be larger in the summer period than in the winter period (see also figure 1). However, this is the opposite of what was found in our study. The reason for this is not clear yet. A possible reason could be that during the winter consumers do froth more milk for beverages than during the summer. The relation between foam properties and season could also be coincidental and it is possible that there is no real correlation between the time of production and foaming properties of the milk. The fact that in our research we did only observe one whole milk sample that has a significant lower foam stability is consistent with the fact there where almost no complaints about the foaming properties of in the period of research.

For semi-skimmed milk there was much more variability in stability than for the whole milk samples. Three samples showed a significant lower stability (HV 25-10-11, 05-12-11 and 29-11-11), while one sample (HV 13-10-11) had a higher stability compared to average. Similarly to the whole milk, no correlation was found between time of production and stability for the semi-skimmed milk.

Furthermore, no significant difference in foam ability and foamstability is observed between foam made with semi-skimmed and whole milk A, as might be seen in figure 5.

In our study, the frothing occurred at temperatures above 40°C. Therefore, because fat crystals were molten, they could not pierce the fat globule membrane and no effect of the fat content on foamability was expected (Huppertz T, 2010). The expectations were in line with our findings. During cooling, fat crystals may form and may have a destabilizing effect on foam stability. However, on the other hand, homogenized fat globules, which are for a large part covered with proteins (Aiqian Y *et al.* 2008), have a foam stabilizing effect (Borcherding, 2007, Huppertz, 2010).

In order to get more insight in possible causes for bad foaming properties, we also measured relevant parameters for foaming behaviour like surface tension, protein content, dissolved casein content, FFA content, and FFA composition.

3.3 Surface tension

Figure 6 shows the foamability and foam stability as a function of the surface tension of (organic) semi-skimmed and whole milk of brand A. The average surface tension of semi skimmed and whole organic milk were similar, respectively 38±3mN/m and 37±4mN/m. It might be expected the average surface tension of whole milk to be higher than that of semi-skimmed milk, due to the higher fat content (Niloshree, 2005) and due to the fact that that milk fat contains more surface active components like, free fatty acids and phospholipids than the serum (Walstra, 2005). Nevertheless, this was not observed in our research.

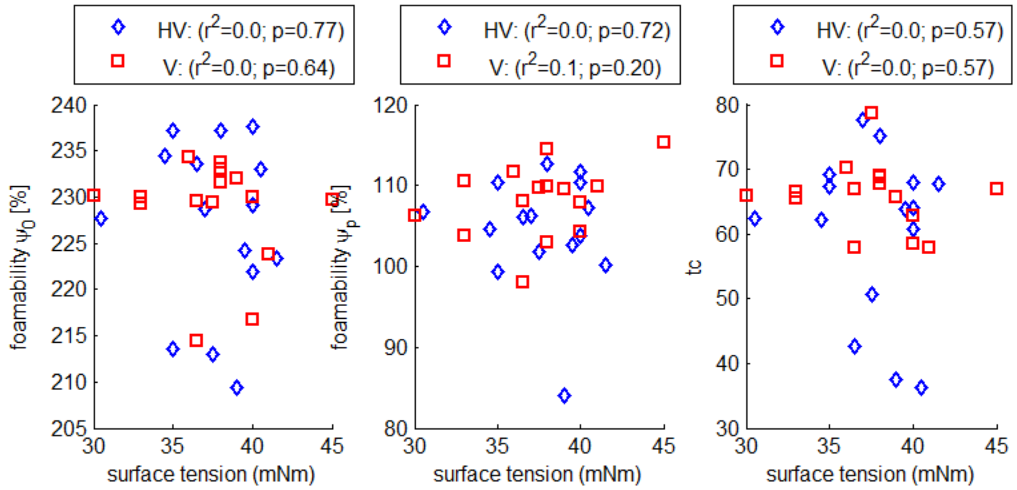

Figure 6. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of the semi-skimmed (HV, blue diamond) and whole (V, red square) milks of brand A (see figure 4) as a function of the measured surface tensions. In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated.

Furthermore, figure 6 shows that there was no correlation between surface tension and both foamability and foam stability. In earlier research a correlation of R^2=0.55 was found (Kamath *et al,* 2008). This is most probably due to the fact that in the research in question raw milk was studied, while we used homogenized pasteurized organic milk.

3.4 Protein content

Figure 7 shows the foamability and foam stability as a function of protein content for organic whole and semi skimmed milk of brand A. The total protein content of semi-skimmed milk was about 3.53%±0.08% (w/w%), and larger than that of whole milk 3.41%±0.08%.

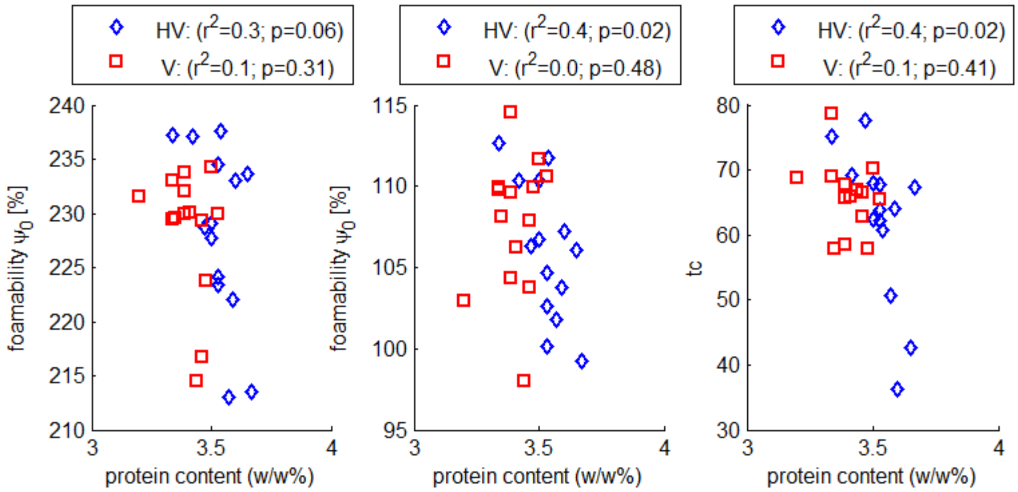

Figure 7. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of the semi-skimmed (HV, blue diamond) and whole (V, red square) A milks (see figure 4) as a function of the measured protein content. In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated.

The difference in protein content between whole and semi skimmed milk was small. Furthermore a very weak (negative) correlation was found between foamability and the protein content as well as for foam stability and protein content, only for semi-skimmed milk. The reason for this is not clear. If there is a correlation one would expect a positive correlation, for both types of milk. Most probably the correlation is not real due to the fact that milk contains an excess of protein (Huppertz, 2010).

3.5 Dissolved casein content

Figure 8 shows the foamability and foam stability of organic semi-skimmed and whole milk of brand A as a function of their content of dissolved casein.

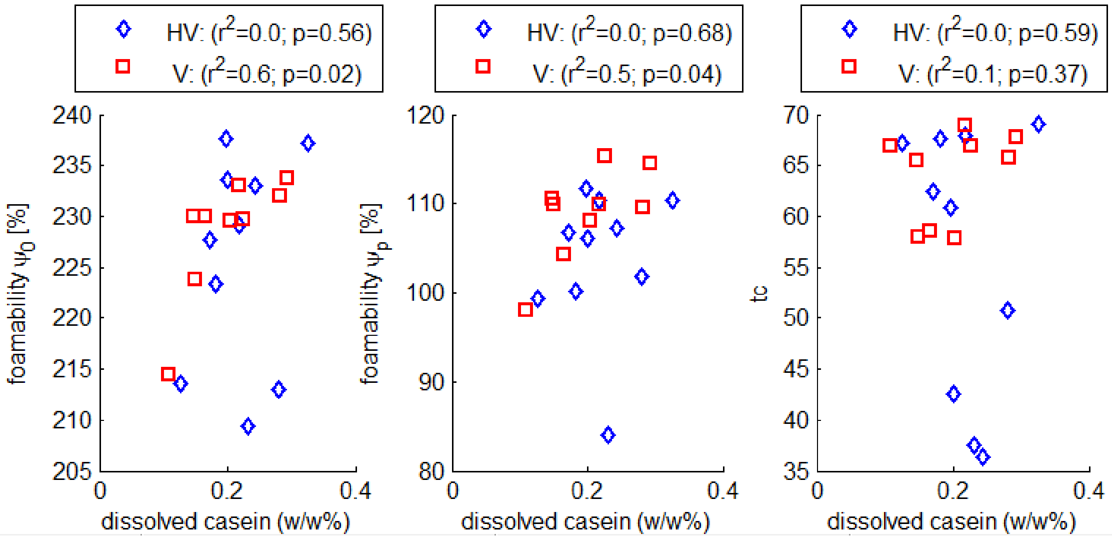

Figure 8. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of the semi-skimmed (HV, blue diamond) and whole (V, red square) A milks (see figure 4) as a function of the measured amount of dissolved casein. In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated.

The average dissolved casein contents and standard deviations were 0.22%±0.05% (w/w%) for semi-skimmed and 0.20%±0.06% (w/w%) for whole milk. It was expected to observe a positive correlation between the concentration of dissolved casein present in milk and its foamability. This because dissolved casein is a very good emulsifier and will bound at the air/water interface.

Interestingly, a weak significant correlation between foamability and the amount dissolved casein was found only for whole milk. For semi skimmed milk, there was no correlation between these two parameters. Furthermore, for both semi-skimmed and full fat milk, no correlation was found between foam stability and dissolved casein content.

The reason why a relation between casein content and foamability was observed only for whole milk is not clear and needs further investigation. Non-structured flexible random coils like β-casein will adsorb rapidly at the air/water interface and will form a viscoelastic surface. Therefore, one might expect a correlation between foamability as well as foam stability and dissolved casein. However, if so, this counts for semi-skimmed as well as for whole milk. Furthermore, as mentioned above, milk has an excess of protein compared to what is necessary for good foam formation (Huppertz, 2010). So no correlation might than be observed after all.

3.6 Fat content

Figure 9 shows the foamability and foam stability of semi-skimmed and whole milk of brand A as a function of fat content. The average fat content and standard deviation of semi-skimmed and whole milk are 1.93%±0.01% (w/w%) and 4.7%±0.1% (w/w%). The difference in fat content did not lead to a difference in foamability and foam stability, as can be seen from the figure. As discussed above, this might be expected for homogenized milk and frothing at a temperature above 40°C.

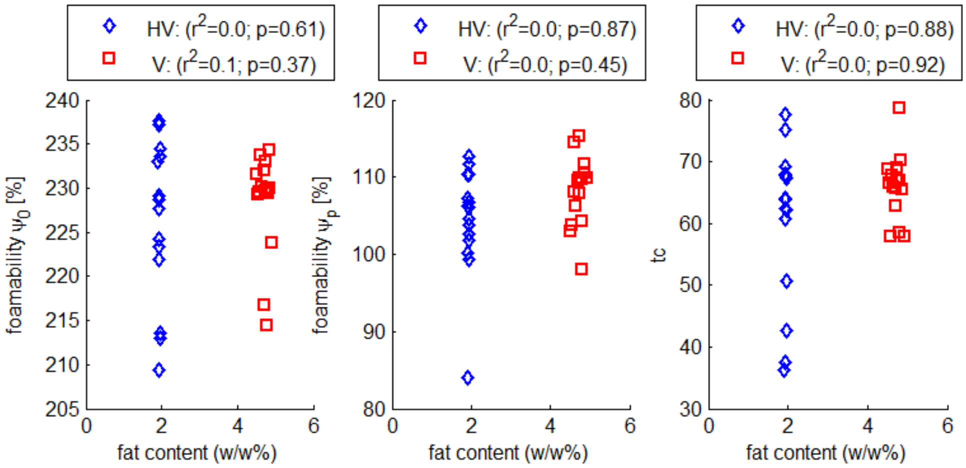

Figure 9. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of the semi-skimmed (HV, blue diamond) and whole (V, red square) A milks (see figure 4) as a function of the measured fat content. In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated.

3.7 Fat globule diameter

Figure 10 shows the foamability and foam stability as a function of the average diameter of the fat globule of semi skimmed milk and whole milk of brand A. The average fat globule diameter for semi skimmed milk was 0.81µm with a standard deviation of 0.06µm. For whole milk 0.87µm±0.06µm was found.

A weak correlation was found between fat globule size and foamability for semi skimmed milk. No significant correlation was found between the foamability and fat globule size for whole A milk as well as between foam stability and fat globule size for both semi-skimmed and whole A milk.

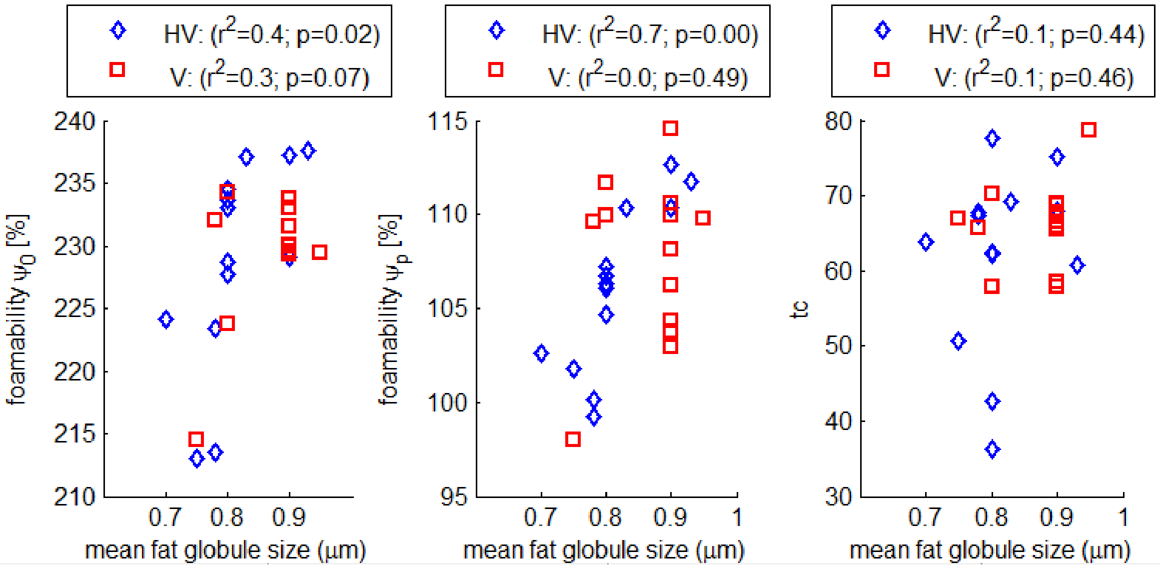

Figure 10. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of the semi-skimmed (HV, blue diamond) and whole (V, red square) A milks (see figure 4) as a function of the measured mean fat globule size. In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated.

These weak correlations could be due to the homogenisation. Before homogenisation the average fat globule diameter is about 4µm (Walstra, 2003). After homogenisation the average diameter is about 4 times smaller (dependently of the homogenisation pressure), implying that the total surface area increases about a factor of 4. The newly formed fat globule membrane is covered directly with milk proteins (Aiqian, 2008). This might result in a lower dissolved protein concentration and, therefore, a slower protein adsorption and thus a lower foamability and foam stability. At first sight, this might be contradictory with respect to the findings that milk has an excess of protein as compared to the amount required for frothing (Huppertz, 2010). However, the experiments were not performed under similar conditions. To be conclusive, the observed weak correlations between fat globule size and foaming properties need more investigation.

3.8 Addition of FFA linoleic acid

It is expected that the amount of FFA has an negative influence on the foaming properties of milk. In order to check this we performed some experiments where we measured the foaming properties of milk to which we added linoleic acid in different amounts for different milk varieties. Experiments were performed in triplicate. Figure 11 shows examples of the measured overrun as a function of time for two different milk varieties. It is seen that for the semi-skimmed A milk the overrun does not follow the characteristic shape anymore for large amount of added linoleic acids. Therefore we also used the half-life (equation 3) as a measure of foam stability. Figure shows the foaming properties of the various varieties

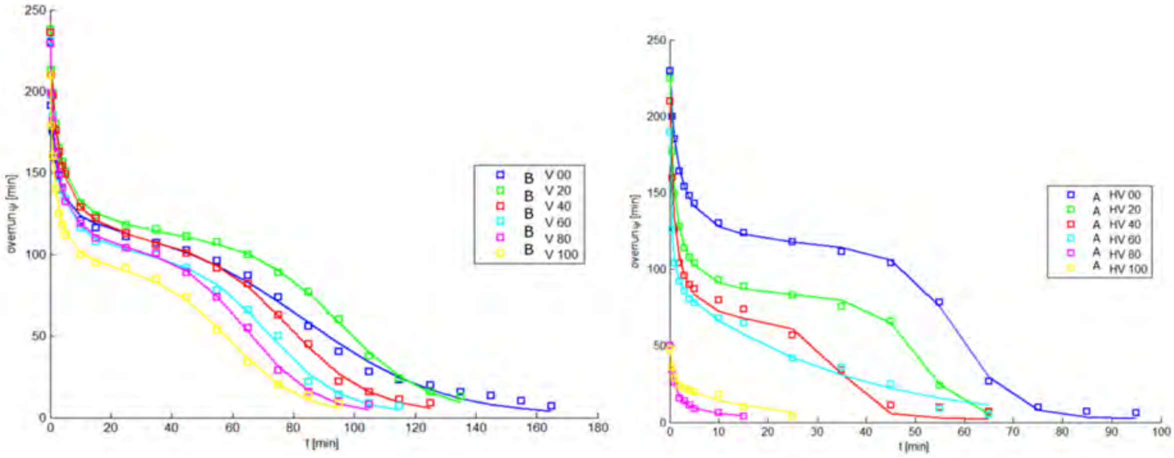

Figure 11. Examples of measured overrun (symbols) of two milk varieties, brand B whole milk (B V, left) and brand A semi-skimmed milk (A HV, right) to which different amounts of linoleic acid are added. The added amounts are indicated in the legend: 00, 20, 40, 60, 80, 100 corresponds to 0, 0.24, 0.47, 0.71, 0.94, and 1.18 mg/l added linoleic acid, respectively.

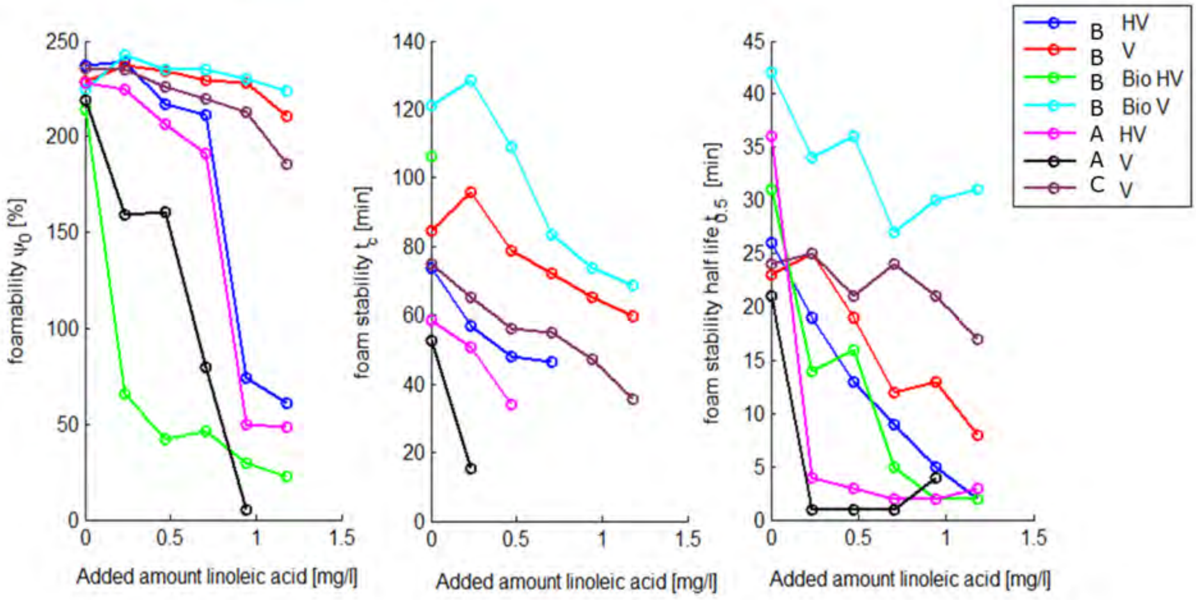

Figure 12. Foamability φ_0 (left) and foam stability, t_c (middle) and half-life $t_{1/2}$ as a function of added linoleic acid for different milk varieties (brand A, B,and C. M= skimmed milk, HV= semi skimmed milk, V=whole milk, Bio=organic milk)

It can be seen that the addition of linoleic acid has a negative effect on the foaming properties of milk. For almost all experiments, the correlation coefficient is above $r^2>0.85$ and significant $p<0.05$. For both semi-skimmed and whole milk of brand A as well as semi-skimmed milk of brand B the addition of linoleic acids had the strongest effect. The other milk varieties show only a small decrease in foamability. Probably the milks that show a stronger decrease in foaming behaviour already had a higher concentration of (long chain) free fatty acids. To check this we measured the FFA content and composition of different milk.

3.9 FFA composition and content

Literature and our results indicate that FFA content have an influence on foaming properties. The question arises if this is due to the short or long chain FFA. Therefore the FFA concentration of C4, C6, C8, C10, C12, C14, C16, and C18 was determined of different semi-skimmed milk samples. Overrun as a function of time is shown in figure 13 while the relation between foaming properties and FFA concentration is shown in figure 14. In the latter figure we only show two typical examples (C10 and C18) as well as the total FFA content (sum of all concentrations). The other measured concentration view similar: no clear and significant correlation between FFA content/composition could be determined.

In earlier research Kamath (2008) found a correlation of 0.6 between foamability and FFA content and a correlation of 0.9 between foam stability and FFA content. The differences with our results are not yet clear yet and need further investigation. They might be attributed to differences in homogenization, sample storage and/or used method to measure FFA content. Here we determined all FFA present.

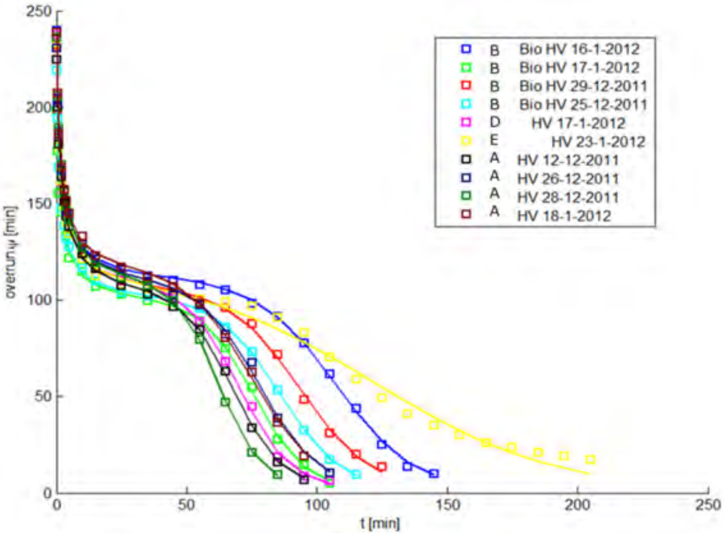

Figure 13. Measured overrun (symbols) as a function of time of various organic milk varieties, of which FFA composition is determined (see figure 14). Also shown are the best fit using equation 2 (continuous lines).

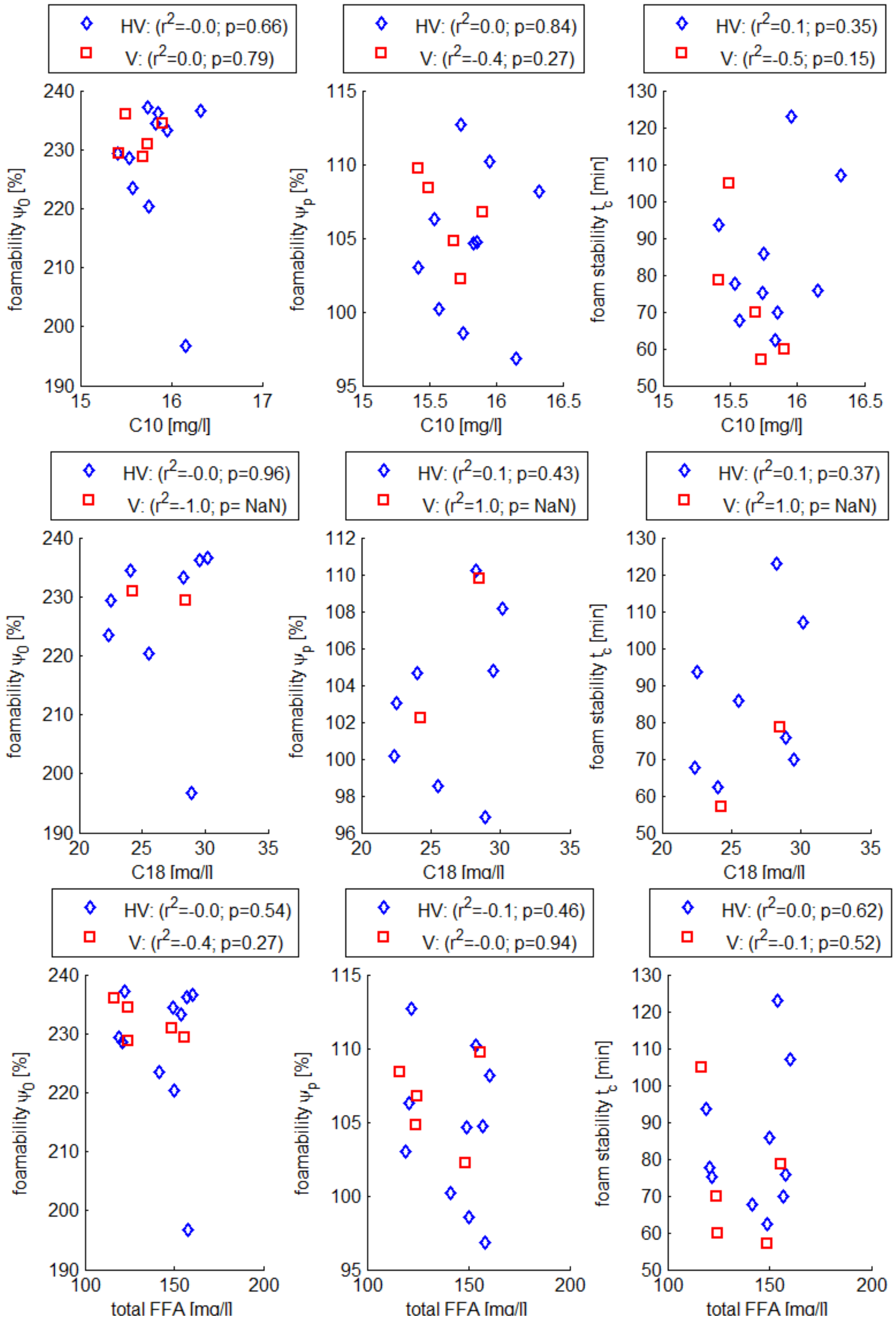

Figure 14. Foamability φ_0 (left) and plateau value φ_p (middle) and foam stability, t_c (right) of different semi-skimmed (HV, blue diamond) and whole (V, red square) organic milks (see figure 13) as a function of the FFA content C10 (top panel), C18 (middle panel) and total sum of C4, C6, C8, C10, C12, C14, C16, and C18 (bottom panel). FFA content of other than C10 and C18 show similar behaviour (data not shown). In the legend the correlation coefficient (r^2) and p-value (indicating significance) are indicated

4. Conclusions

The foaming properties of different commercial milk varieties were tested. The foamability of the studied milks turned out to be similar, while the foam stability differed between different varieties. No clear relation could be observed between foam stability and fat content (skimmed vs. semi-skimmed vs. full-fat) and/or type (regular vs. organic). However, foams made from milk of brand A, the focus of our study, showed the lowest stability of all tested milk varieties. The reason for this is not yet clear, but the bad foam stability of this milk might be probably due to a higher free fatty acid content. However, we could not demonstrate that in this study.

The foaming properties of semi-skimmed and whole A milk purchased in the local stores of Wageningen, the Netherlands, in the period from September 2011 till January 2012 were studied. Surface tension, protein content, dissolved casein content, fat content, average fat globule size and free fatty acid content and composition were determined and compared to the foaming properties.

Only one whole and two semi-skimmed organic milk samples were observed that showed a lower foam stability than average, in line with the low number of complaints in this period. No relation in foaming properties and difference in season (meadow/summer versus stable/winter period) could be observed. Whole A milk seemed to give more stable foams than semi-skimmed A milk.

No correlation was found between surface tension and foaming properties. Also no correlation was found between protein content and foaming properties. For dissolved casein only a weak correlation with foamability was observed for whole milk and not for semi-skimmed milk. The reason is not clear yet and needs further investigation.

No correlation is found between the fat content and foaming properties, although the fat content of whole milk was significantly larger than that of semi skimmed milk. This is most probably because the foam is frothed at 56°C, a temperature at which all fat crystals are melted. Than they cannot pierce the fat globule membrane and release liquid fat that could cause foam instability.

A weak correlation was found between the fat globule diameter and foamability for semi skimmed milk. Very weak correlations were found between fat globule size and both foamability of whole milk and foam stability of semi skimmed milk. No correlation was found between the foam stability and fat globule size for whole A milk.

It is expected that foaming properties are negatively influenced by free fatty acids (FFA). This was supported by our experiments where we added small amount of linoleic acid (C18) to various milk varieties. However, we also determined FFA content and composition of different milks. In these experiments, no correlation was observed between free fatty acids content and foamability. The reason for this is no clear and also needs further investigation.

Although there are indications that the amount of free fatty acids is negatively related to foaming properties of milk, no clear cause for the occasionally bad foaming properties of organic milk could yet be identified. This needs further investigation One of the main reasons for this outcome is probably that only very few milk samples were found with significant worse foaming properties. Another reason might be that the studied samples were commercial bulk milk of which the history concerning technological and heat-treatments was unknown. For a better mechanistic understanding of the role of milk composition on foaming properties both the temperature and process history should be known or controlled.

References

- Aiqian Y, Skelte G Anema and Harjinder Sinh (2008), Changes in the surface protein of the fat globules during homogenization and heat treatment of concentrated milk. Journal of Dairy Research **75** 347-353
- Akerlind M, Holtenius K, Bertilsson J, Emanuelson M (1999), Milk composition and feed intake in dairy cows selected for high or low fat percentage. Swedish dairy association
- Bos M. A. and T. van Vliet (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a revieuw. Adv Colloid interface Sci 91(3): 437-471
- Borcherding K, Hofman W, Lorenzen P Chr and Schrader K. (2008) Effect of milk homogenisation and foaming temperature on properties and microstructure of foams from pasteurized whole milk. *Lebensmitteln Wissenschaft und Technologie* **41** 2036-2043
- Borcherding K, Lorenzen P Chr., Hoffmann W, Schradeer K., (2007) Effect of foaming temperature and varying time/temperature-conditions of pre-heating on the foaming properties of skimmed milk. International Dairy Journal **18** (2008) 349-358
- Buchanan R A. (1965) Lipolysis and the frothing of milk. *The Australian Journalof Dairy Technology* **20** 62-22
- Brooker B.E., Anderson M. and Andrews A.T., The development of structure in whipped cream. Food Microstructure, **5** (1986), pp. 277-285
- Cempirkova R., Mikulova M., (2009) Incidence of psychrotrophic lipolytic bacteria in cow’s raw milk. Czech J. Anim. Sci., **54** 65-73
- Chandan, R. Dairy Based Ingredients: Practical Guides for the Food Industry, Eagen Press Handbook Series, USA, 1997
- Chilliard Y., Glasser F., Ferlay A., Bernard L., Rouel J., Doreau M. (2007) Diet rumen biohydrogenation and nutritional quality of cow and goat milk fat. Journal Lipid science technology **109** 828-855
- Chouinard P. Y., Louise Corneau, David M. Barbano, Lioyd E. Metzger and Dale E. Bauman (1999) Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. Animal science and food science, cornell university, Ithaca, NY 14853
- Cornec M. Mackie A.R., Wilde P.J. Clarck D.C, Colloid. Surface A-Physicochemical. Eng. Aspects 114 (1996) 237.
- Couvreur S., Hurtaud C., Lopez C., Delaby L., Peyraud J.L (2006). The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition and butter properties. J. Dairy Sci., 89: 1956-1969
- Cumper, C. W. (1953) *Trans Farad. Soc*. **49**, 1360.
- Deeth H C and Smith R A D (1983) Lipolysis and other factors affecting the steam frothing capacity of milk. *The Australian Journal of Dairy Technology **38** 14-19*

- Deeth H C. (2002) Lipolyses in milk and dairy products – A research journey. Food Australia **10** 433-436
- Dewhurst R.J., Fisher W.J., Tweed J.K.S., Wilkins R.J. (2003). Comparison of grass and legume silage for milk production. Production response with different levels of concentrate. Journal Dairy Science **86** 2598-2611
- Dhiman T. R., Satter L. D., Pariza M. W., Galli M. P. Albright K. and Tolosa M. X., (2000) Conjugated Linoleic Acid (CLA) Content of milk from cows offerd diets rich in linoleic and linolenic acid. Journal Dairy Science **83** 1016-1027
- Dickinson E (1989) Adsorption at interfaces in dairy systems. Journal of the Dairy Technology, Vol. 42, No. 1, February 1989
- Dickinson E (1999) Adsorbed protein layers at fluid interfaces: interactions, structure and surface reology, Projector Department of food Science, University of Leeds
- Dickinson E. (2010) Mixed biopolymers at interfaces: Competetive adsorption and multilayer structures. Food hydrocolloids **25** 1966-1983
- Ferlay A., Martin B., Pradel Ph., Coulon J.B., Chilliard Y. (2006). Influence of grass based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. J. Dairy Sci., 89: 4026-4041
- Graham D. & Phillips M. (1975) *Foams,* (Ackers, R. J. (Ed)), Academic press, London, 237
- Gustafson M, Salomon E, Jonsson S, Steineck S, (2003) Fluxes of K, P, and Zn in a conventional and anorganic dairy farming system through feed, animals, manure, and urine. European journal of argonomy **20** 88-89
- Hohe K. A., Dimick P. S., and Kilara A. (1985) Milk lipoprotein lipase distribution in the major fractions of bovine milk. Journal of dairy science **68** 1067-1073
- Holden T F, Aceto N C, Dellamonica E S and Calhoun M J (1966) Seasonal varietion of phospholipids and their influence on the foaming characteristics of co ncentrated whole milk. *Journal of Dairy Science* **49** 346-350
- Hupprtz T. (2010) Foaming properties of milk: A revieuw of the influence of composition and processing. International Journal of Dairy Technology **63**, 477
- Huppertz T. and Kelly A. L. (2006) Physical chemistry of milk fat globules. In Advanced dairy chemistry 2: Lipids, pp. 173-212
- IDF, Determination of free fatty acids in milk & milk products. IDF Bulletin, No. 265. Brussels, Belgium: International Dairy Federation. 1991
- Jiang J., Bjoerck L., Fonden R. and Emanuelson M., Occurence of conjugated cis-9, trans-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. Journal Dairy Science **79** 438-445

- Kamath S. (2008) Relationship between surface tension, free fatty acid concentration and foaming properties. Food research international 41 623-629
- Kamath S, Huppertz T, Houlihan A V and Deeth H (2008) The influence of temperature on the foaming of milk. International Journal **18** 994-1002
- Kelly M. L., Berry J. R., Dwyer D. A., Griinari J. M., Chouinard P. Y., Van Amburgh M. E., Bauman D. E. (1998) Dietary fatty acids sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. Journal Nutrition 881-885
- Kinsella J E (1981) Functional properties of proteins: possible relationship between structure and function in foams Food Chemistry **7** 273-288
- Mackie A.R., Gunning A.P, Wilde P.J., Morris V.J. LanGmuir 16 (2000) 2243
- Meyers S. A, Cuppett S. L., and Hutkins R. W. (1996) , Lipase production by lactic acid bacteria and activity on butter oil. Food Microbiology **13** 383-389
- Molkentin J and Giesmann A, (2007) Anal Bioanal Chem 388:297-305
- Mukherjee N.and Bansal, B. and Chen, Xiao Dong (2005) Measurement of surface Tension of Homogenised Milks. **1**
- Niloshree M, Bipan B, Xiao Dong Chen, 2005 Measurement of Surface Tension of Homogenised milks. International Journal of Food Engineering. **1** pp. 1-6
- Nilsson-Ehle, P., *et al.* Positional specificity of purified Milk Lipoprotein Lipase. J. Biol. Chem., 1973. **248** p:6734-6737
- Parodi P. W., Conjugated octadecadienoic acid of milk fat. Journal Dairy science, **60** 1550-1553
- Prins A. (1986) Some physical aspects of aerated milk products. Netherlands Milk and Dairy Journal 40 203-215
- Prins A. (1999) Stagnant surface behaviour and its effect on foam and film stability. Colloids and surfaces **149** 467-473
- Ruis M. & Pinxterhuis J. B. (2007), Verantwoorde en communiceerbare argumenten bij biologische producten: dierenwelzijn. Animal Sciences Group 35-65
- Schroder M., Yousefi F., Vetter W., (2010) Investigating the day-to-day variations of potential marker fatty acids for organic milk in milk from conventionally and organically raised cows 167-174
- Walstra P (2003) Physical Chemistry of food. New York: Marcel Dekker Inc.
- Wilde P, Mackie A, Husband F, Gunning P and Morris V (2004) Proteins and emulsifiers at liquid interfaces. *Advanced in Colloid and Interfacial Science* **108** 63-71
- Zhang Z, Goff H. D. (2003) Protein distribution at air interfaces in dairy foams and ice cream as affected by casein dissociation and emulsifiers. International Dairy Journal **14** 647-657

Bioconnect aims to continue developing and reinforcing organic agriculture by initiating and executing research projects. Within Bioconnect, entrepreneurs (from farmers to shop owners) collaborate with educational and research institutions as well as consultants. This results in a demand-driven approach, which is unique in Europe.

Bioconnect
Het innovatienetwerk van biologische agroketens

The Dutch Ministry of Economy, Agriculture and Innovation is financing the research projects.

Ministerie van Economische Zaken,
Landbouw en Innovatie

The Wageningen UR (University & Research centre) and the Louis Bolk Institute carry out the research. Actually, there are about 120 research projects for biological agriculture.

LOUIS BOLK
INSTITUUT

WAGENINGEN UR
For quality of life

www.biokennis.nl

Market & Chains