

**PRODUCTION OF TRADITIONAL INDIAN BUTTER
(MAKKHAN): PROCESS IMPROVEMENT
AND QUALITY ASSESSMENT**

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INSTITUTE OF TECHNOLOGY DELHI**

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by

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CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

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CERTIFICATE

This is to certify that the thesis entitled, “**Production of Traditional Indian Butter (Makkhan): Process Improvement and Quality Assessment**” being submitted by **Mr. Kumaresh Halder** to the **Indian Institute of Technology Delhi** for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of thesis. To the best of our knowledge, the results contained in the thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

(Prof Jatindra K Sahu)

Centre for Rural Development & Technology
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(Prof S N Naik)

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ABSTRACT

Makkhan is a very popular and widely consumed traditional cultured fat rich milk product of the Indian subcontinent. According to Food Safety and Standards Regulations (FSSR), *makkhan* should contain at least 76% milk fat by weight. *Makkhan* is prepared by fermentation of whole milk or *malai* (i.e. clotted cream) into curd (i.e. *dahi*) followed by churning the curd into aggregates of crystallized fat. *Makkhan* contains a higher proportion of high melting fraction of milk fat, which is believed to enrich its flavour and texture. In spite of potential health benefits, wide consumption pattern, and significant commercial importance, the scientific study on characteristics of *makkhan* is rarely available. Conventional production practices lead to greater amount of fat loss in buttermilk (i.e. *chhach*) with low volume of *makkhan* production. Therefore, improvement in the production of raw material such as cream containing size differentiated milk fat globules (MFGs) with higher production volume would be a better option to bring improvement in *makkhan* production. With this background, the present research was designed to study the detail characteristics of *makkhan* available in households, to develop a process for production of *makkhan* from creams with size differentiated MFGs, and to study its impact on quality characteristics and storage properties of the product for large scale production.

A comparative study was made between fresh cow and buffalo milk *makkhan* samples procured from households at different locations in India. The samples were analyzed in terms of the proximate composition, microbiological, thermal, rheological, flavour and sensory characteristics. To develop a process for the production of *makkhan* using creams with size differentiated MFGs, the inside geometry of a commercial small scale cream separator (60 L.h⁻¹) was modified to vary the channel gaps between the conical discs as 1, 1.5 and 2 mm. Then the values for disc spacing, bowl speed and feed rate were selected to achieve a cut-off diameter (d_c) i.e 3 μ m of MFGs in creams using a MATLAB programme followed by experimental validation using a Box–Behnken Design of experiments. Further selection of values for process parameters were accomplished to minimize the number of small MFGs (< 3 μ m) in creams (> 25% fat) by evaluating the performance of a selected value of disc spacing at different combinations of bowl speed and feed rate. For performance evaluation of the modified centrifuge the performance parameters such as MFGs size, fat content, and yield of cream samples were measured. Production of creams using a two-stage modified separation approach was further adopted to minimize MFGs with diameter less than 3 μ m in creams (> 25% fat) and compared with cream produced using the unmodified cream separator. For the comparative analysis, MFGs size, fat content, fatty acid composition and thermal properties of cream samples were determined. Before fermentation, cream samples were standardized to 28-30% fat, heated to 92-95°C for 15s and immediately cooled to 30°C. Inoculation of cream samples was done with an activated mesophilic lactic acid bacteria (LAB) NCDC-193 @ 2% of cream and incubated at 30°C for 10 h. Samples were analysed at 0 and 10 h for total lactic count, pH and titratable acidity. Churning was conducted at two temperatures i.e. 13°C and 19°C. The parameters like churning time, fat loss in buttermilk, fat content and yield of *makkhan* were measured to evaluate the churning process. Impact of creams with size differentiated fat globules in comparison with whole milk on *makkhan* production using the selected protocol was evaluated in terms of quality attributes of *makkhan*. For storage studies, *makkhan* samples were stored at refrigeration (5°C) and frozen (-18°C) storage conditions. The samples were evaluated at 3rd, 15th and 30th day of storage for their physico-chemical properties, flavour profile, acid and peroxide values, and sensory characteristics.

There were slight variations between cow milk *makkhan* (CMM) and buffalo milk *makkhan* (BMM)

samples. CMM samples contained higher saturated fat, while BMM samples were found to contain higher conjugated linoleic acid (CLA). Both *makkhan* samples showed solid-like behaviour at 22°C. Colour of the CMM sample was found more acceptable by the sensory panel. For production of cream with size based fractionated fat globules in the modified separator, a combination of 1.5 mm disc spacing, 750 mL.min⁻¹ feed rate and 4500 rpm bowl speed was found as optimum condition to achieve a cut-off diameter (d_c) of 3 µm. From the experiments the value of disc spacing was selected as 1.5 mm for modified separator and after its performance evaluation with six different combinations of process parameters a feed rate 700 mL.min⁻¹ was found most suitable in combination with bowl speed of 4000 and 4500 rpm. For two-stage cream separation process, cream produced from the first stage was designated as cow partially separated cream (CPSC) and that produced from second stage was designated as cow washed cream (CWC) contained mean fat globules size of 4.16±0.07 µm and 4.77±0.03 µm respectively from whole cow milk with average fat globule size of 3.52±0.09 µm. Similarly, buffalo partially separated cream (BPSC) and buffalo washed cream (BWC) produced with average fat globule size of 4.93±0.16 µm and 5.18±0.16 µm respectively from whole buffalo milk with average size of 3.76±0.12 µm. CWC and BWC contained higher proportion of large milk fat globules (LMFGs), higher solid fat and greater proportion of saturated fat such as stearic acid (C18:0) compared to cow fully separated cream (CFSC) and buffalo fully separated cream (BFSC) respectively. The fermentation of cream samples with lactic culture NCDC-193 indicated that there was approximately 1.2 fold increment in bacterial count, approximately 3.5 to 4.0 fold increment in acid production and approximately 1.3 fold reduction in pH value in each sample. Churning of each fermented cream at 13°C was observed to take slightly longer time for churning compared to 19°C. Churning of fermented CWC and BWC at 13°C prevented fat loss in buttermilk significantly, and *makkhan* produced with significantly higher yield and increased fat recovery compared to other creams. It was observed that cow washed cream *makkhan* (CWCM) and buffalo washed cream *makkhan* (BWCM) samples contained improved proximate composition and microbiological properties than other *makkhan* samples. Quantity of diacetyl flavour was found to be slightly lower in CWCM and BWCM samples (905.49±149.07 ppb and 840.48±182.69 ppb respectively) than other samples, but those were more acceptable according to the sensory panel. CWCM and BWCM contained higher amount of health beneficial components such as CLA (1.00±0.22 and 1.04±0.01%) and lower amount of saturated fatty acid (SFA) (65.00±0.65 and 65.57±0.67%) compared to other *makkhan* samples. The presence of approximately 20-40% solid fat in all *makkhan* samples indicated that spreadability of the samples could be acceptable. The viscoelastic properties revealed that the values of storage (G') and loss (G'') moduli were moderate in CWCM and BWCM, and both the samples were not found to be very hard or not very soft in texture and possessed good spreadability at 22°C. There was no significant change in physico-chemical and microbial properties of CWCM and BWCM samples, but titratable acidity and oxidative stability in the samples stored at refrigeration condition were found to increase at relatively faster rate compared to frozen storage. More amount of flavour was accumulated in the samples stored at frozen storage.

In conclusion, the present study validated the few important claims regarding presence of CLA, textural attributes, thermal properties and flavouring components made earlier. The developed process for production of *makkhan* from washed creams with size differentiated fat globules brought significant improvement in quality attributes of *makkhan*, and that process could be recommended for large scale production of *makkhan*.

Keywords: *Makkhan*, whole milk, modified centrifugal separation, cream, milk fat globules size, quality attributes, storage properties

सारांश

मक्खन भारतीय उपमहाद्वीप का एक बहुत लोकप्रिय और व्यापक रूप से खाया जाने वाला पारंपरिक सुसंस्कृत वसा युक्त दूध उत्पाद है। खाद्य सुरक्षा और मानक विनियम (एफएसएसआर) के अनुसार, मक्खन में वजन के हिसाब से कम से कम 76% दूध वसा होना चाहिए। मक्खन शुद्ध दूध या मलाई (यानी क्लॉटेड क्रीम) को दही में किण्वन द्वारा तैयार किया जाता है, जिसके बाद दही को क्रिस्टलीकृत वसा के समुच्चय में मथा जाता है। मक्खन में दूध वसा के उच्च पिघलने वाले अंश का उच्च अनुपात होता है, जिसके बारे में माना जाता है कि यह इसके स्वाद और बनावट को समृद्ध करता है। संभावित स्वास्थ्य लाभ, व्यापक खपत पैटर्न और महत्वपूर्ण व्यावसायिक महत्व के बावजूद, मक्खन की विशेषताओं पर वैज्ञानिक अध्ययन नितन्त सीमित है। पारंपरिक उत्पादन प्रक्रियाओं से छाछ में अधिक मात्रा में वसा की हानि होती है और मक्खन के उत्पादन की मात्रा बहुत कम होती है। इसलिए, उच्च उत्पादन मात्रा के साथ आकार विभेदित दूध वसा ग्लोब्यूल्स (एमएफजी) के साथ क्रीम जैसे कच्चे माल के उत्पादन में सुधार, मक्खन उत्पादन में सुधार लाने का एक बेहतर विकल्प होगा। इस पृष्ठभूमि के साथ, वर्तमान शोध को घरों में उपलब्ध मक्खन की विस्तृत विशेषताओं का अध्ययन करने, आकार में विभेदित एमएफजी के साथ क्रीम से मक्खन के उत्पादन के लिए एक प्रक्रिया विकसित करने और बड़े पैमाने पर उत्पादन, उत्पाद की गुणवत्ता विशेषताओं और भंडारण गुणों पर इसके प्रभाव का अध्ययन करने के लिए डिज़ाइन किया गया था।

भारत में विभिन्न स्थानों पर घरों से प्राप्त ताजा गाय और भैंस के दूध के मक्खन के नमूनों के बीच एक तुलनात्मक अध्ययन किया गया था। नमूनों का विश्लेषण समीपस्थ संरचना, सूक्ष्मजैविक, ऊष्मीय, रियोलॉजिकल, स्वाद और संवेदी विशेषताओं के संदर्भ में किया गया था। आकार विभेदित एमएफजी के साथ क्रीम का उपयोग करके मक्खन के उत्पादन के लिए एक प्रक्रिया विकसित करने के लिए, एक व्यावसायिक छोटे पैमाने के क्रीम सेपरेटर (60 लीटर प्रति घंटा) की आंतरिक ज्यामिति को शंकाकार डिस्क के बीच चैनल अंतराल को 1, 1.5 और 2 मिमी के रूप में बदलने के लिए संशोधित किया गया था। फिर डिस्क स्पेसिंग, बाउल स्पीड और फ्रीड रेट के मूल्यों को एक कट-ऑफ व्यास (डीसी) प्राप्त करने के लिए चुना गया था, यानी MATLAB प्रोग्राम का उपयोग करके क्रीम में एमएफजी के 3 माइक्रोन, इसके बाद प्रयोगों के बॉक्स-बेहेनकेन डिज़ाइन का उपयोग करके प्रायोगिक सत्यापन किया गया। कटोरे की गति और फ्रीड दर के विभिन्न संयोजनों पर डिस्क रिक्ति के चयनित मूल्य के प्रदर्शन का मूल्यांकन करके क्रीम (> 25% वसा) में छोटे एमएफजी (< 3 माइक्रोन) की संख्या को कम करने के लिए प्रक्रिया मापदंडों के मूल्यों का आगे चयन पूरा किया गया। संशोधित सेंट्रीफ्यूज के प्रदर्शन मूल्यांकन के लिए एमएफजी आकार, वसा सामग्री और क्रीम के नमूनों की उपज जैसे प्रदर्शन मानकों को मापा गया। क्रीम में 3 माइक्रोमीटर (> 25% वसा) से कम व्यास वाले एमएफजी को कम करने के लिए दो-चरण संशोधित पृथक्करण दृष्टिकोण का उपयोग करके क्रीम का उत्पादन और असंशोधित क्रीम विभाजक का उपयोग करके उत्पादित क्रीम के साथ तुलना की गई। तुलनात्मक विश्लेषण के लिए, एमएफजी आकार, वसा सामग्री, फैटी एसिड संरचना और क्रीम के नमूनों के थर्मल गुण निर्धारित किए गए थे। किण्वन से पहले, क्रीम के नमूनों को 28-30% वसा के लिए मानकीकृत किया गया था, 15 s के लिए 92-95 डिग्री सेल्सियस तक गरम किया गया और तुरंत 30 डिग्री सेल्सियस तक ठंडा किया गया। क्रीम के नमूनों का टीकाकरण एक सक्रिय मेसोफिलिक लैक्टिक एसिड बैक्टीरिया (एलएबी) एनसीडीसी-193 @ 2% क्रीम के साथ किया गया था और 10 घंटे के लिए 30 डिग्री सेल्सियस पर ऊष्मायन किया गया था। कुल लैक्टिक काउंट, पीएच और टिट्रेटेबल एसिडिटी के लिए 0 और 10 घंटे में नमूनों का विश्लेषण किया गया। मंथन दो तापमानों यानी 13 डिग्री सेल्सियस और 19 डिग्री सेल्सियस पर व्यावस्थित किया गया था। मंथन की प्रक्रिया का मूल्यांकन करने के लिए मंथन का समय, छाछ में वसा की कमी, वसा की मात्रा और उत्पन्न मक्खन की मात्रा जैसे मापदंडों को मापा गया। मक्खन के गुणवत्ता गुणों के संदर्भ में चयनित प्रोटोकॉल का उपयोग करके मक्खन उत्पादन पर पूरे दूध की तुलना में आकार विभेदित वसा वाले ग्लोब्यूल्स के प्रभाव का मूल्यांकन किया गया था। भंडारण अध्ययन के लिए, मक्खन के नमूनों को प्रशीतन (5°C) और हिमशीतित (-18°C) भंडारण स्थितियों में संग्रहीत किया गया था। नमूनों का उनके भौतिक-रासायनिक गुणों, स्वाद प्रोफ़ाइल, एसिड और पेरोक्साइड मूल्य, और संवेदी विशेषताओं के भंडारण के तीसरे, 15वें और 30वें दिन मूल्यांकन किया गया था।

गाय के दूध के मक्खन (सीएमएम) और भैंस के दूध के मक्खन (बीएमएम) के नमूनों में मामूली अंतर था। सीएमएम के नमूनों में उच्च संतृप्त वसा थी, जबकि बीएमएम के नमूनों में उच्च संयुग्मित लिनोलिक एसिड (सीएलए) पाया गया। मक्खन

के दोनों नमूनों ने 22 डिग्री सेल्सियस पर ठोस जैसा व्यवहार दिखाया। संवेदी पैनल द्वारा सीएमएम नमूने का रंग अधिक स्वीकार्य पाया गया। संशोधित विभाजक में आकार आधारित फ्रैक्शनेटेड फैट ग्लोब्यूल्स के साथ क्रीम के उत्पादन के लिए, 1.5 मिमी डिस्क स्पेसिंग, 750 एमएल प्रति मिनट फीड रेट और 4500 आरपीएम बाउल स्पीड का संयोजन कट-ऑफ व्यास (डीसी) 3 माइक्रोन प्राप्त करने के लिए इष्टतम स्थिति के रूप में पाया गया। प्रयोगों से संशोधित विभाजक के लिए डिस्क स्पेसिंग के मूल्य को 1.5 मिमी के रूप में चुना गया था और प्रक्रिया मापदंडों के छह अलग-अलग संयोजनों के साथ इसके प्रदर्शन मूल्यांकन के बाद 700 एमएल प्रति मिनट की फीड दर 4000 की बाउल गति के साथ संयोजन में सबसे उपयुक्त पाई गई थी और 4500 आरपीएम। दो चरणों वाली क्रीम पृथक्करण प्रक्रिया के लिए, पहले चरण से उत्पादित क्रीम को गाय आंशिक रूप से अलग क्रीम (सीपीएससी) के रूप में नामित किया गया था और दूसरे चरण से उत्पादित गाय की धुली हुई क्रीम (सीडब्ल्यूसी) के रूप में नामित किया गया था जिसमें $4.16 \pm 0.07 \mu\text{m}$ और $4.77 \pm 0.03 \mu\text{m}$ का माध्य वसा ग्लोब्यूल्स था। $3.52 \pm 0.09 \mu\text{m}$ के औसत वसा ग्लोब्यूल्स आकार के साथ पूरे गाय के दूध से क्रमशः इसी तरह, भैंस के आंशिक रूप से अलग किए गए क्रीम (बीपीएससी) और भैंस की धुली हुई क्रीम (बीडब्ल्यूसी) का उत्पादन पूरे भैंस के दूध से क्रमशः $4.93 \pm 0.16 \mu\text{m}$ और $5.18 \pm 0.16 \mu\text{m}$ के औसत वसा वाले ग्लोब्यूल्स आकार के साथ $3.76 \pm 0.12 \mu\text{m}$ के औसत आकार के साथ किया जाता है। सीडब्ल्यूसी और बीडब्ल्यूसी में बड़े दूध वसा वाले ग्लोब्यूल्स (एलएमएफजी), उच्च ठोस वसा और संतृप्त वसा जैसे स्टीयरिक एसिड (C18: 0) का उच्च अनुपात गाय पूरी तरह से अलग क्रीम (सीएफएससी) और भैंस पूरी तरह से अलग क्रीम (बीएफएससी) की तुलना में होता है। क्रमशः, लैक्टिक कल्चर एनसीडीसी -193 के साथ क्रीम के नमूनों के किण्वन ने संकेत दिया कि प्रत्येक नमूने में बैक्टीरिया की संख्या (लॉगसीएफयू प्रति एमएल) में लगभग 1.2 गुना वृद्धि, एसिड उत्पादन में लगभग 3.5 से 4.0 गुना वृद्धि और पीएच मान में लगभग 1.3 गुना कमी थी। 13 डिग्री सेल्सियस पर प्रत्येक किण्वित क्रीम को मथने में 19 डिग्री सेल्सियस की तुलना में थोड़ा अधिक समय लगता है। 13 डिग्री सेल्सियस पर किण्वित सीडब्ल्यूसी और बीडब्ल्यूसी को मंथन करने से छाछ में वसा की कमी काफी हद तक कम हो गई, मक्खन का उत्पादन अन्य क्रीमों की तुलना में काफी अधिक मिला और वसा प्राप्ति में वृद्धि हुई। यह देखा गया कि सीडब्ल्यूसीएम बीडब्ल्यूसीएम के नमूनों में अन्यमक्खन के नमूनों की तुलना में बेहतर समीपस्थ संरचना और माइक्रोबियल गुण थे। अन्य नमूनों की तुलना में सीडब्ल्यूसीएम और बीडब्ल्यूसीएम नमूनों ($905.49 \pm 149.07 \text{ ppb}$ और $840.48 \pm 182.69 \text{ ppb}$ क्रमशः) में डायसेटाइल स्वाद की मात्रा थोड़ी कम पाई गई, लेकिन वे संवेदी पैनल के अनुसार अधिक स्वीकार्य थे। सीडब्ल्यूसीएम और बीडब्ल्यूसीएम में अन्य मक्खन के नमूनों की तुलना में सीएलए (1.00 ± 0.22 और $1.04 \pm 0.01\%$) जैसे स्वास्थ्य लाभकारी घटकों की उच्च मात्रा और संतृप्त फैटी एसिड (एसएफए) की कम मात्रा (65.00 ± 0.65 और $65.57 \pm 0.67\%$) शामिल हैं। सभी मक्खन के नमूनों में लगभग 20-40% ठोस वसा की उपस्थिति ने संकेत दिया कि नमूनों की फैलाव क्षमता स्वीकार्य हो सकती है। viscoelastic गुणों से पता चला है कि सीडब्ल्यूसीएम और बीडब्ल्यूसीएम में भंडारण (G') और हानि (G'') मोडुली के मूल्य मध्यम थे, और दोनों नमूने बनावट में बहुत कठोर या बहुत नरम नहीं पाए गए और 22 °C पर अच्छी प्रसार क्षमता रखते थे। सीडब्ल्यूसीएम और बीडब्ल्यूसीएम नमूनों के भौतिक-रासायनिक और माइक्रोबियल गुणों में कोई महत्वपूर्ण परिवर्तन नहीं हुआ था, लेकिन प्रशीतन स्थिति में संग्रहीत नमूनों में टिट्रेटेबल अम्लता और ऑक्सीडेटिव स्थिरता जमे हुए भंडारण की तुलना में अपेक्षाकृत तेज दर से बढ़ी हुई पाई गई। जमे हुए भंडारण में संग्रहीत नमूनों में अधिक मात्रा में स्वाद जमा हुआ था।

उपसंहार के रूप में, वर्तमान अध्ययन ने स्वास्थ्य लाभकारी घटकों जैसे सीएलए, टेक्सचरल विशेषताओं, थर्मल गुणों और पहले किए गए फ्लेवरिंग घटकों की उपस्थिति के संबंध में कुछ महत्वपूर्ण तथ्यों (दावों) को वैज्ञानिक मान्यता दी। अलग-अलग आकार के फैट ग्लोब्यूल्स के साथ धुली हुई क्रीम से मक्खन के उत्पादन के लिए विकसित प्रक्रिया ने मक्खन की गुणवत्ता विशेषताओं में महत्वपूर्ण सुधार लाया, इस प्रकार उस प्रक्रिया को मक्खन के बड़े पैमाने पर उत्पादन के लिए अनुशंसित किया जा सकता है।

Key words: मक्खन, शुद्ध दूध, संशोधित केन्द्रापसारक पृथक्करण, क्रीम, दूध वसा ग्लोब्यूल्स का आकार, गुणवत्ता गुण, भंडारण गुण

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LIST OF ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
≥	Greater than or equal to
@	At the rate
AI	Atherogenic Index
ANOVA	Analysis of variance
DAHD	Department of Animal Husbandry & Dairying
FSSAI	Food Safety and Standards Authority of India
FSSR	Food Safety and Standards Regulations
NDRI	National Dairy Research Institute
NCDC	National Collection of Dairy Cultures
AOAC	Association of Official Analytical Chemists
IS	Indian Standard
°C	Degree Celsius
CLA	Conjugated linoleic acid
d_c	Cut-off diameter
DSC	Differential scanning calorimeter
Eq.	Equation
FA	Fatty acid
FAME	Fatty acid methyl esters
Fig.	Figure
FFA	Free fatty acid
FSC	Fully separated cream
g	gram
g.L^{-1}	gram per litre
G'	Storage modulus
G''	Loss modulus
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectroscopy
h	Hour

HPLC	High Performance Liquid Chromatography
HSD	Honestly Significant Difference
ISO	International Organization for Standardization
IDF	International Dairy Federation
kg	Kilogram
kg.m ⁻³	Kilogram per meter cube
kg.s ⁻¹	Kilogram per second
LAB	Lactic acid bacteria
LCFA	Long chain fatty acid
LMFG	Large milk fat globule
LMF	Low melting fraction
cfu.mL ⁻¹	Colony forming unit per mili Litre
µm	micrometer
µL	micro Litre
MCFA	Medium chain fatty acid
mm	milimeter
m/z	mass to charge ratio
min	minute
meq	mili equivalent
MFG	Milk fat globule
MMFG	Medium milk fat globule
MMF	Medium melting fraction
mL	mili Litre
mL.min ⁻¹	mili Litre per minute
mg	mili gram
MRS	De Man, Rogosa and Sharpe
MUFA	Monounsaturated fatty acid
MS	Mass spectroscopy
°C.min ⁻¹	Degree Celsius
O ₂	Oxygen
Pa	Pascal
Pa.s	Pascal second
ppb	Parts per billion

ppm	Parts per million
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
R^2	Co-efficient of determination
rpm	Revolution per minute
rps	Revolution per second
rad.s ⁻¹	Radian per second
s	Second
SCFA	Short chain fatty acid
SD	Standard deviation
SMFG	Small milk fat globule
SNF	Solids not fat
SPME	Solid phase micro extraction
SPE	Solid phase extraction
TAG	Triacylglycerol
TI	Thrombogenic index
V	Volt
v	volume
VLCFA	Very long chain fatty acid
w	weight
W	Watt
