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Conjugated linoleic acid-rich chocolate paste production and characterization

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ABSTRACT

Conjugated linoleic acid (CLA) is an 18-carbon fatty acid with multiple health benefits, including anti-obesity and anti-carcinogenic properties. CLA-rich soy oil (CLARSO) can be produced through a heterogeneous catalysis process, and this oil was previously used to produce CLA-rich margarines and shortenings. The objective of this study was to produce CLA-rich chocolate pastes by replacing a portion of the fat with CLARSO and compare the rheological (flow), textural, and thermal properties of these pastes to controls made with either soy oil or traditional fats. CLARSO was used to prepare pastes. Rheology, firmness, and thermal behavior of the pastes were determined. The CLARSO chocolate pastes contained no additional saturated fat relative to soy oil controls but the pastes had more solid-like rheology and were firmer. Relative to non-soy controls, CLARSO pastes had similar rheology, despite containing less saturated fat. The fat crystals of all samples were in the same polymorphic form. Therefore, it was successfully demonstrated that CLARSO has the ability to produce chocolate pastes with similar physical properties as traditional products containing more saturated fat.

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MEET THE STUDENT-AUTHOR

Sarah Mayfield

I am from San Antonio, Texas and graduated from Smithson Valley High School in 2011. I spent 4 years at the University of Arkansas studying Food Science and Biochemistry and graduated in the spring of 2015. This fall, I will be pursuing a Ph.D. in Food Science at the University of Arkansas under the direction of Dr. Andy Proctor.

During my undergraduate career, I was president of the Food Science Club, a Bumpers College Student Ambassador, and a Co-Chair for the American Oil Chemists' Society Student Division. I had the opportunity to travel to the University of Gent in Ghent, Belgium during the summer of 2014 to conduct a portion of my honors thesis research. In addition to providing me with valuable research experience, this taught me the importance of inter-institutional collaboration, particularly in an international setting.

I would like to thank Dr. Andy Proctor for providing me the opportunity to conduct research under his supervision and for serving as an excellent research mentor. I would also like to thank the University of Gent, and in particular Dr. Koen Dewettinck and Dr. Ashok Patel, for allowing me to work in their laboratories and for helping me with numerous manuscript edits. Lastly I would like to thank all of the institutions that have provided funding for this project: the Dale Bumpers College, the University of Arkansas Honors College, the Arkansas Department of Higher Education SURF Grant, and the University of Gent.

INTRODUCTION

Conjugated linoleic acid (CLA) is an 18-carbon dietary fatty acid found mostly in dairy and bovine foods (Whigham et al., 2000). Conjugated linoleic acid has many positive human health benefits, including anti-carcinogenic properties (Cesano et al., 1998; Kim et al., 2002), and the ability to fight atherosclerosis (Feitoza et al., 2009; Nicolosi et al., 1997), lower the risk of diabetes (McGuire and McGuire, 1999), and improve immune function (Gilbert et al., 2011). However, the typical diet provides only a fraction of the 3.2 grams of CLA needed daily to gain these health benefits, and increasing dietary CLA by increased consumption of bovine foods is unadvisable as they are also high in saturated fat and cholesterol (Ip et al., 1991; Mougios et al., 2001). Therefore, a CLA-rich food source that is low in saturated fats and cholesterol would be desirable.

Soy oil contains approximately 50% linoleic acid (LA), a fatty acid with the potential to be isomerized to CLA, and could therefore be used to produce CLA-rich food products that are low in saturated fat and cholesterol (Gangidi and Proctor, 2004). Jain and Proctor (2006) developed a process to produce a 20% CLA-rich soy oil (CLARSO) through the photoisomerization, or the excitation of molecules by light to induce a structural change, of soy oil LA in the presence of an iodine catalyst. However, it was necessary to remove the iodine in this process, which therefore made it difficult to commercialize. Shah and Proctor (2013) developed a heterogeneous catalysis process that produces 20% CLARSO in 2 hours without the use of solvents. A ruthenium-on-carbon catalyst is combined with soy oil in a high temperature, vacuum distillation technique, similar to the deodorization process routinely used in industry. Unlike the iodine-catalyzed photoisomerization process (Jain and Proctor, 2006), the ruthenium catalyst easily can be centrifuged and filtered out of the oil (Yettella et al., 2012).

Ruan and Proctor (2014) found that CLARSO had greater solid fat properties relative to conventional soy oil, including increased viscosity and a higher melting point temperature. A CLARSO margarine was developed by Shah et al. (2014) and the firmness, rheology (flow behavior), thermal behavior, and microstructure were compared to a soy oil control and a commercially available margarine. The CLARSO margarine was firmer, better able to tolerate high levels of stress without deformation, and had a higher solid fat content than the soy oil control, while having physical properties comparable to those of commercial margarine. Five typical servings of this mar-

garine would provide the recommended daily value of CLA and 185 Calories. Mayfield et al. (2015) subsequently developed CLA-rich shortenings and compared their rheology, thermal properties, and microstructure to those of soy oil controls and commercially available shortenings. The CLA-rich shortenings possessed more solid-like rheological properties and had a more stable crystal structure, as indicated by differential scanning calorimetry (DSC) analysis, than did the soy oil controls. Furthermore, CLA shortenings also had similar physical properties to commercial controls. The results of the margarine and shortening studies illustrate the effectiveness of CLARSO as a replacement for conventional saturated fats in food products, while providing additional health benefits.

Chocolate, like shortening, is a fat-based food whose physical properties are dependent upon its polymorphic crystal structure. Therefore, the oils and fats used to produce chocolate products have a significant effect on product quality. It will be of interest to know how such a replacement of palm oil with CLARSO in chocolate paste will affect chocolate products.

Therefore, the objective of this study was to determine the functional physical properties of chocolate paste prepared by replacing 25% of a palm oil/canola oil mixture with CLARSO, relative to control pastes obtained by replacing 25% of the palm/canola mixture with soy oil, and a control made solely with the palm/canola oil mixture.

MATERIALS AND METHODS

Conjugated Linoleic Acid-Rich Soy Oil Production and Analysis

The heterogeneous catalytic process of Shah and Proctor (2013) was adopted to produce CLA-rich soy oil from refined, bleached, deodorized (RBD) soy oil (Riceland Foods, Stuttgart, Ark., USA), which was used to produce chocolate pastes and bars. The method of Lall et. al. (2009) was used to determine the fatty acid profile of RBD and CLARSO duplicate samples as fatty acid methyl esters (FAMES). Each sample was analyzed by gas chromatography with a flame ionization detector (GC-FID).

Chocolate Paste Production and Analysis

Chocolate Paste Preparation. Chocolate pastes were prepared according to the method of Patel et al. (2014). There were three types of chocolate paste produced: CLARSO, soy oil control, and non-soy oil control. Each paste contained 30% (wt.) of a fat blend, the composition of which differed based on the type of paste. The fat blend used for the control paste consisted of 70% palm oil and 30% canola oil (Vandemoortele R&D, Izegem, Belgium). The fat blends used for the CLA-rich and soy oil pastes were prepared by making the control fat blend (70% palm oil and 30% canola oil) and then replacing 25% of this with either CLARSO or soy oil, so that these final fat blends contained 52.5% palm oil, 22.5% canola oil, and 25% of either CLARSO or soy oil.

One kilogram of each type of chocolate paste was prepared with these fat blends, as described in Table 1. Eighty percent (wt/wt) of the fat blend and Palsgaard Oil Binder (Palsgaard A/S, Denmark) were combined in a Stephan UMC 5 mixer (Stephan Machinery, Hameln, Germany) set to a temperature of 60 °C and stirred until the Oil Binder was completely dissolved. Milk powder (Friesland, Campina, Belgium), cocoa powder (Cargill, Wormer, The Netherlands), and crushed sugar (Barry Callebaut, Wieze, Belgium) were then added to the mixture which was stirred for approximately 2 min. The particle size of the mixture

Table 1. Composition of conjugated linoleic acid-rich oil, soy oil, and control chocolate pastes.

	CLARSO paste		Soy oil paste		Control paste	
Ingredient	% By mass	Amount (g)	% By mass	Amount (g)	% By mass	Amount (g)
Palm oil	15.75	157.5	15.75	157.5	21.0	210.0
CLARSO	7.5	75.0	---	---	---	---
Soy oil	---	---	7.5	75.0	---	---
Canola oil	6.75	67.5	6.75	67.5	9.0	90.0
Palsgaard Oil Binder	1.50	15.0	1.50	15.0	1.50	15.0
Crushed sugar	47.85	478.5	47.85	478.5	47.85	478.5
Skim milk powder	14.0	140.0	14.0	140.0	14.0	140.0
Cocoa powder	6.0	60.0	6.0	60.0	6.0	60.0
Palsgaard PGPR 4150	0.15	1.5	0.15	1.5	0.15	1.5
Palsgaard AMP 4448	0.5	5.0	0.5	5.0	0.5	5.0

was reduced through refining using an Exakt 80S 3-roll mill (Exakt Technologies Inc., USA) with a roll temperature of 35 °C, a roll distance of 3-1, and a speed of 400 rpm. The refined mixture was placed back into the Stephan Mixer, the remaining fat blend, Palsgaard PGPR 4150, and Palsgaard AMP 4448 (Palsgaard A/S, Denmark) were added, and the mixture was stirred for approximately 2 min. The chocolate paste was transferred into 20 cylindrical plastic containers (1 in. diameter × 1 in. height) for texture analysis and into 6-50-mL centrifuge tubes for rheology and thermal analysis. Pastes were stored at 20 °C for one week prior to analysis.

Rheology Determination. The rheology of the pastes was determined in sample duplicate after one week of storage at 20 °C (deemed "week 1") and after two weeks of storage at 20 °C (deemed "week 2") with an AR 2000 Rheometer (TA Instruments, New Castle, Del., USA). Strain and frequency sweeps were performed to determine gel strength and viscoelastic behavior of the samples. A cross-hatched parallel plate geometry (diameter = 40 mm) was used with a geometry gap set at 1000 μm. The strain sweep involved increasing the strain from 0.0001 to 100 while keeping the temperature constant at 20 °C. The complex modulus G' was measured as a function of strain to analyze the resistance of the samples to deformation as there was increased stress applied to the sample. The frequency sweep involved increasing the frequency from 0.1 to 100 Hz while keeping the temperature at 20 °C.

Firmness Analysis. Firmness analysis was performed on chocolate paste samples after one and two weeks of storage at 20 °C. Five sample replicates were analyzed using a 5942 Instron TA 500 Texture Analyzer (Lloyd Instruments, Bognor Regis, West Sussex, UK). Firmness was defined as the force required to penetrate the samples using an 11 mm diameter cylindrical probe which entered the samples to a depth of 10 mm at a rate of 10 mm/min with a 0.1 N trigger value.

Thermal Analysis. Fat blends as used in each chocolate paste sample were prepared. The control blend contained 70% palm oil and 30% canola oil; the CLARSO blend contained 52.5% palm oil, 22.5% canola oil, and 25% CLARSO; and the soy oil blend contained 52.5% palm oil, 22.5% canola oil, and 25% soy oil. The melting behavior of triplicate samples was determined by differential scanning calorimetry (DSC) using a Q1000 Tzero DSC (TA Instruments, New Castle, Del., USA). Indium (enthalpy and temperature), azobenzene (temperature), and undecane (temperature) were used to calibrate the instrument. The instrument was purged with nitrogen and an empty pan was used as reference. Five to ten mg of each fat blend was placed in hermetic aluminum pans and the pans were sealed. The melting behavior of the samples was determined by equilibrating the pans at 20 °C for 10 min and then increasing the temperature at a rate of 5 °C/min to a final temperature of 70 °C. The heat flow was measured as a function of temperature and the resulting DSC curves were analyzed using the Universal Analysis software (TA Instruments, New Castle, Del.). A single inverted peak (endothermic peak) was observed and four parameters of this peak were analyzed: the onset temperature, the temperature at which the maximum heat flow was observed (the peak temperature), the offset temperature, and the enthalpy absorbed (the peak integration).

Data Analysis

All statistical analyses were performed using JMP 10 (SAS Institute, Inc., Cary, N.C.) statistical software. The fatty acid composition, rheology, texture, and thermal results were analyzed by comparing the overall means in a one-way analysis of variance using Tukey's honest significant difference test with an α-level of 0.05. The strain and frequency sweep G' values were transformed logarithmically to obtain a better comparison, as the data .

RESULTS AND DISCUSSION

Conjugated Linoleic Acid-Rich Soy Oil Fatty Acid Analysis

There was no significant difference between the total saturated fat content of CLARSO and soy oil, as shown in Table 2. The CLARSO contained a total saturated fat content of 18.74% and the soy oil contained a total saturated fat content of 18.09%. The CLARSO contained 20.83% CLA. Therefore, 15.4 g of this oil would need to be consumed daily in order to receive the recommended 3.2 g of CLA.

Table 2. Fatty acid data for conjugated linoleic acid-rich soy oil (CLARSO) and soy oil used to produce chocolate pastes and bars.

Fatty Acid†	CLARSO %	Soy Oil %
C16:0	13.54 ± 0.03a‡	13.98 ± 0.70a
C18:0	5.20 ± 0.03a	4.112 ± 0.80b
C18:1	26.78 ± 0.03a	23.25 ± 1.22b
C18:2	31.85 ± 0.03b	54.74 ± 1.58a
C18:3	1.80 ± 0.01b	5.41 ± 0.80a
CLA *cis,trans/trans,cis*	15.76 ± 0.41	-
CLA *trans,trans*	5.07 ± 0.41	-
Total CLA	20.83 ± 0.00	-
Total saturated fat	**18.74 ± 0.04a**	**18.09 ± 1.07a**

† Samples were analyzed in duplicate and error indicates standard deviation. Statistical analysis was performed to identify significant differences between individual fatty acids.

‡ Samples connected by same letter are not significantly different.

Table 3. Overall mean G' values for one decade of strain (0.01-0.1) from the strain sweep analysis of conjugated linoleic acid-rich, soy oil, and control chocolate paste samples.

Sample	Overall mean G'†
Control week 1	15.98 ± 0.22 a‡
CLARSO week 1	14.98 ± 0.14 c
Soy oil week 1	13.69 ± 0.30 d
Control week 2	15.97 ± 0.21 a
CLARSO week 2	16.02 ± 0.28 a
Soy oil week 2	15.51 ± 0.25 b

† Analysis was performed in duplicate and error indicates standard deviation. The means were transformed logarithmically to obtain a better comparison.

‡ Samples with the same connecting letter are not significantly different.

Table 4. Overall mean G' values from the frequency sweep analysis of conjugated linoleic acid-rich, soy oil, and control chocolate paste samples.

Sample	Overall mean angular frequency (rad/s)†
Control week 1	15.87 ± 0.22a‡
CLARSO week 1	13.53 ± 0.17de
Soy oil week 1	13.50 ± 0.18e
Control week 2	15.65 ± 0.27b
CLARSO week 2	15.30 ± 0.21c
Soy oil week 2	12.95 ± 0.21f

† Analysis was performed in duplicate and error indicates standard deviation. The means were transformed logarithmically to obtain a better comparison.

‡ Samples with the same connecting letter are not significantly different.

Chocolate Paste Characterization

Rheology Determination. The mean G' values for one decade of % strain, from 0.01 to 0.1, were compared to identify any statistically significant differences in solid-like behavior among samples (Table 3). For both the CLARSO and soy oil samples, the week 2 samples had significantly higher G' values than their respective week 1 values. Therefore, post-production isothermal crystallization and hardening occurred in both of these samples, increasing their solid-like behavior. There was no significant difference between the control week 1 and week 2 samples. The CLARSO week 1 and week 2 samples had significantly higher mean G' values than the soy oil week 1 and week 2 samples, respectively. This indicated that CLARSO provided the chocolate paste with a more solid crystal structure, without the addition of saturated fats. The CLARSO week 2 sample was not significantly different from the control week 2 sample. The control sample contained a greater portion of solid fat but had solid behavior similar to that of the CLARSO sample. Therefore, CLARSO behaved more like a solid fat without contributing additional saturated fatty acids.

The overall mean G' values were compared to determine any statistically significant differences among samples in angular frequency (Table 4). Similar to the strain sweep analysis, this was an indication of solid behavior.

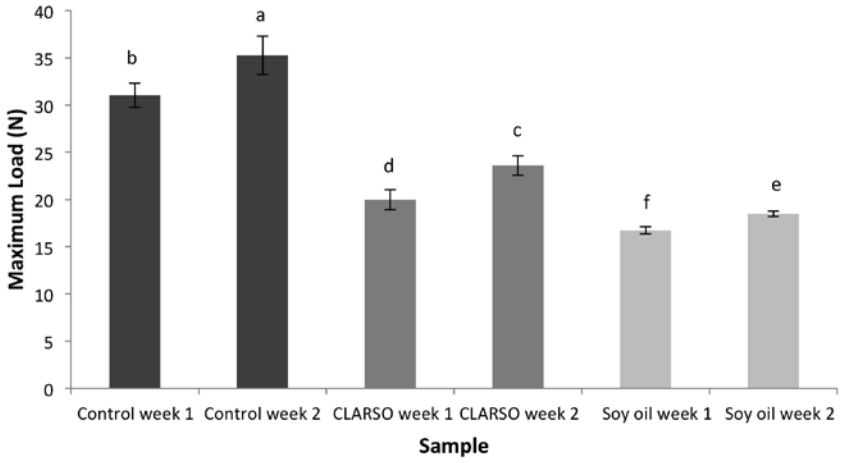

Fig. 1. Bar graph displaying the mean maximum load measured during firmness analysis of conjugated linoleic acid-rich, soy oil (CLARSO), and control chocolate pastes. Measurements were taken after one and two weeks of storage at 20 °C. Five sample replicates were analyzed for each type of paste and error bars indicate standard deviation. Samples with the same connecting letter are not significantly different.

The CLARSO week 2 sample had a significantly higher mean G' value than did the CLARSO week 1 sample. However, the control and soy oil week 2 samples had significantly lower mean G' values than did their respective week 1 samples. Therefore, the CLARSO sample displayed post-production isothermal crystallization hardening in this case but the control and soy oil samples did not. Although the CLARSO and soy oil week 1 samples were not significantly different, the CLARSO week 2 sample had a significantly higher G' value than did the soy oil week 2 sample. This was consistent with the strain sweep results: the CLARSO provided a more solid-like structure without contributing additional saturated fats.

Firmness Analysis

Figure 1 shows the maximum force achieved when samples of CLARSO, soy oil, and control chocolate pastes were penetrated with a cylindrical probe, as an indication of the firmness of the samples. All week 2 samples showed significantly greater firmness than their respective week 1 samples. This indicates that significant amounts of post-production isothermal crystallization/hardening occurred, which is consistent with the results of the strain sweep analysis. Both the CLARSO week 1 and week 2 samples were significantly firmer than their respective soy oil samples. This was attributed to the ability of CLARSO to provide the samples with a more solid-like structure without the addition of saturated fats, as also seen in the rheology results. The control week 1 and week 2 samples were significantly firmer than the respective CLARSO and soy oil samples, indicating that although CLARSO offered improved texture over soy oil, it did not behave similarly to the more saturated palm oil with regards to texture.

Thermal Analysis

Table 5 shows the values and statistical comparisons for the onset temperature, peak temperature, offset temperature, and enthalpy of the CLARSO, soy oil, and control chocolate paste fat blends. There were no significant differences in melting onset temperatures among any of the samples. This indicated that the fat crystals were in the same polymorphic form in all of the samples (Braipson-Danthine and Deroanne, 2004). There was no significant difference between the peak melting temperature of the CLARSO and soy oil fat blends. However, the control sample had a significantly higher peak temperature. This result was expected due to the greater amount of saturated fat in the control fat blend (Damodaran et al., 2007). There were no significant differences in offset temperature among any of the samples, indicating that their most saturated fraction, the palm oil, finished melting at the same temperature, as was expected. No significant differences in enthalpy were observed among any of the samples. This result illustrated that all samples had similar levels of thermodynamic stability and similar solid-like crystal matrices (Himawan et al., 2006). Addition of CLARSO did not significantly affect the thermodynamic stability and solid behavior of the sample relative to the control fat blend. This is consistent with the strain sweep results and again indicated the potential of CLARSO to replace more saturated fats.

The typical serving size for chocolate pastes is 1 Tablespoon, or approximately 40 g (U.S. FDA, 2014). To obtain the recommended daily value of CLA (3.2 g), it would be necessary to consume 5 servings of chocolate paste.

CONCLUSIONS

Although the CLARSO paste contained no additional solid fats in relation to the soy oil paste, it displayed more solid-like physical properties. The CLARSO paste had more solid rheological and textural properties relative to the soy oil control. Therefore, CLARSO provided samples with a more solid crystalline matrix without actually containing additional saturated fat.

Thermal analysis indicated that all samples displayed the same polymorphic crystal form, despite containing different fat blends. Therefore, partial replacement of palm oil with CLA-rich and conventional soy oils did not change the form in which the fats crystallized.

The CLARSO paste displayed similar physical properties relative to the control paste. The rheology and crystalline thermodynamic stability of the CLARSO paste was similar to that of the control paste. Therefore, the solidity of the fat crystal matrices of the CLARSO and control chocolate pastes were similar despite the control samples

Table 5. Differential scanning calorimetry measurements from the melting analysis of conjugated linoleic acid-rich, soy oil (CLARSO), and control chocolate pastes.

Sample	Onset Temp. (°C)	Peak Temp. (°C)	Offset Temp. (°C)	Enthalpy (J/g)
CLARSO fat blend	29.46 ± 0.04a†	42.36 ± 0.06b	44.87 ± 0.07a	4.04 ± 0.24a
Soy oil fat blend	29.60 ± 0.10a	42.43 ± 0.16b	44.93 ± 0.14a	3.89 ± 0.20a
Control fat blend	29.47 ± 0.13a	43.13 ± 0.23a	44.77 ± 0.35a	4.42 ± 0.28a

† Analysis was performed in triplicate and error indicates standard deviation. Samples with the same connecting letter are not significantly different.

containing more solid fats than the CLARSO samples. This demonstrated the ability of CLARSO to replace more saturated fats without compromising solid behavior.

The CLARSO chocolate pastes demonstrated commercialization potential because they had comparable quality to the control pastes and had the ability to provide the recommended daily value of CLA in 5 servings.

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