Effect of pressure-assisted thermal sterilization on conjugated linoleic acid (CLA) content in CLA-enriched milk

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Abstract

Conjugated linoleic acid (CLA) has been recently studied for its health-promoting and disease-preventing properties. The objective of this study was to evaluate the effect of pressure (100-600 MPa), temperature (60-120˚C) and treatment time (0-14 min) on CLA content in milk and anhydrous milk fat (AMF) rich in CLA. In milk treated up to 14 min at 100 MPa, CLA was stable (> 80 % of retention), regardless of the temperature. In contrast, only 3.4 ± 2 % of CLA was retained at 600 MPa and 120°C. For AMF, CLA retention was considerably higher (40.2 ± 2 %) than that obtained for milk. The presence of free metal ions in milk might catalyze CLA degradation. When the antioxidant catechin (1 g/kg) was used, CLA retention increased significantly (> 90%) in milk and AMF, regardless of the experimental conditions.

Keywords*:* conjugated linoleic acid; enriched milk, catechin, anhydrous milk fat, pressure-assisted thermal sterilization.

Industrial relevance*:* Previous research led by Dr. John Kennelly resulted in CLA-enriched milk by manipulating the diet of dairy cattle. CLA in milk suffers significant losses in terms of its biological activity during processing at high temperatures like UHT. High consumption of CLA products has been associated with many health benefits. This study provided new data on CLA retention with and without an antioxidant at pressure-assisted thermal sterilization (PATS) conditions and confirmed the role of catechin in reducing oxidative damage by PATS treatments. Milk rich in CLA can be treated with PATS to deliver shelf-stable milk while CLA activity is preserved.

1. Introduction

Bovine milk contains functional compounds with biological activities that are lost during sterilization treatments (Claeys, Van Loey & Herndrickx, 2002). Among these compounds, conjugated linoleic acid (CLA) has shown potential health benefits, such as cancer prevention, atherosclerosis, weight control, and bone formation (Lock & Bauman, 2004). Epidemiological studies have correlated positively CLA intake with health-promoting and disease-preventing properties (Fritsche et al., 1999). Although these relationships are still under investigation, CLA has shown relevant biological activity (Cook & Pariza, 1998). Dairy products contain the highest amount of CLA in the human diet. In addition, CLA concentration in milk can be markedly enhanced through diet manipulation and management of dairy cattle nutrition (Kennelly and Bell, 2004). Unfortunately, CLA is not stable during thermal processing and significant losses of biologically active CLA occur through oxidation (Campbell, Drake, & Larick, 2003; Herzallah, Humeid, & Al-Ismail, 2005) and isomerization (Destaillats & Angers, 2005).

Pressure-assisted thermal sterilization (PATS) is a new technology that simultaneously applies high pressure (~600-700 MPa) and high temperature (~90-120°C) to inactivate bacterial spores (Mathys, Reineke, Heinz, & Knorr, 2009; Leadley, Tucker, & Fryer, 2008). This technology was first successfully developed to produce sterile low-acid foods commercially (Sizer, Balasubramaniam, & Ting, 2002). In PATS treatments, when the pre-heated product is pressurized, the adiabatic heating is used to reach the target or sterilization temperature (Valdez-Fragoso et al., 2011)(Ting, Balasubramaniam, & Raghubeer, 2002). Although non-uniform temperature profile in high pressure vessels

was reported for industrial units with a temperature variation of ~10 ^{o}C (Knoerzer et al., 2010), the rapid heating in PATS processes reduces the severity of thermal treatment and the lack of temperature uniformity that occurs in traditional sterilization processes (Torres, & Velazquez, 2005; Torres, Sanz, Otero, Pérez Lamela, & Saldaña, 2009; Knoerzer, Buckow, Versteeg, 2010).

Pressure alters interatomic distances, acting mainly on weak interactions in which bond energy is distance–dependent, such as van der Waals forces, electrostatic forces, hydrogen bonding and hydrophobic interactions of proteins. Based on the distance dependence, any pressurized sample would have its covalent bonds intact, thus preserving the biological activity of functional compounds, such as ascorbic acid (Oley, Verlinde, Hendrickx, & Van Loey, 2006), folates (Butz et al. 2004), vitamins (Matser, Krebbers, van den Berg, & Bartels, 2004), and anthocyanins (Verbeyst, Oey, Van der Plancken, Hendrickx, & Van Loey, 2010). These findings along with the PATS inactivation kinetics of bacterial spores (Bartlett, 2002; Margosch et al. 2006; Paredes-Sabja, Gonzalez, Sarker, & Torres, 2007) are promising results, suggesting that this novel technology can deliver shelf-stable milk with better quality than the traditional sterilization process; however, stability of CLA in milk at PATS conditions has not been studied. Therefore, the objective of this study was to evaluate the effect of pressure-assisted thermal sterilization on CLA retention in enriched milk and enriched anhydrous milk fat and the possibility to enhance its retention by the addition of the antioxidant catechin.

2. Materials and Methods

2.1. Enriched milk and anhydrous milk fat (AMF)

Enriched milk was obtained from the University of Alberta Dairy Unit after providing the dairy cattle an appropriate diet following a similar methodology as proposed by Bell, Griinari, & Kennelly (2006) with some modifications. Briefly, 12 lactating Holstein cows were first fed with a control diet for three days. Then, the cows were fed for 16 days with a diet supplemented with sunflower ground seed at 6 % of dry matter. The diet consists of 60 % forage and 40 % concentrate. Cows were housed in tie stalls and water was available at all times. CLA-enriched milk was collected and stored at -20^{o}C until needed.

For experiments that required AMF, the enriched milk was prepared as described earlier by Martinez-Monteagudo, Saldaña, & Kennelly (2012). Briefly, the enriched milk was heated to 55˚C and centrifuged at 6000 x *g* for 6 min using an Alpha-Laval centrifuge (LAPX 202, Lund Scania, Sweden). The cream obtained was stirred for 20 min at room temperature using a hand Black Decker Power Pro Mixer and the obtained butter was washed with cold water to remove excess of butter milk. The butter was heated at 60˚C for 120 min to separate into two layers. The top layer was removed and poured through cheese cloth to obtain AMF rich in CLA. This fat was stored at -18˚C until further analysis.

2.2. CLA and fatty acid determination

CLA content and fatty acids were analyzed by gas-chromatography (GC) according to the methodology described elsewhere (Cruz-Hernandez et al., 2004). The GC (Varian 3400, Varian Inc., Walnut Creek, CA) instrument was equipped with an automated cool on-column injection, a flame-ionization detector, an autosampler, and a fused-silica capillary column (SP-2560, 100 m length × 0.25 mm ID, Supelco Inc., Belfonte, PA). Treated milk (2.7 mL) was mixed with 20 mL of chloroform/methanol solution (ratio of 2:1 v/v). After 1h, 8 mL of 0.9 % of sodium chloride was added. The mixture was centrifuged at 839 x *g* for 5 min and the lower phase (chloroform+lipids) was transferred to a test tube. The extracted lipids were dried with nitrogen at a flow rate of 10 L/min at 30˚C. Base-catalyzed methylation was used to obtain fatty acid methyl esters (FAME). The dried lipids were mixed with 2 mL of hexane, 40 µL of methylacetate, and 80 µL of sodium methoxide (0.5 N) at 60˚C for 20 min. After incubation time, 2 mL of hexane, 2 mL of water and 1 mL of internal standard were added. The internal standard was prepared by dissolving 0.05 g of methyl heptadecanoate (C17:0, Fluka #51633, purity of 99.5%) in 50 mL of hexane. The upper phase of the solution containing the FAME was transferred to GC vials and diluted with hexane at 0.4 mg/mL. Samples of 1 µL of FAME solution were injected under a hydrogen flow rate of ~1 mL/min. The injector and detector temperature was 250˚C. The injected samples were heated at 45˚C for 4 min, then heated to 175˚C at 13˚C/min and held at this temperature for 27 min, then heated to 215˚C at 4˚C/min and held at this temperature for 35 min (Kramer, Cruz-Hernandez, & Zhou, 2001). The chromatograms were analyzed with the Galaxi software V1.19 and the peaks were identified by comparison with a GC reference FAME standard (463-Nu Check Prep., Inc., Elysian, MN).

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143 **2.3. Pressure-assisted thermal sterilization (PATS) treatments**

144 PATS experiments were conducted using a high pressure multivessel system
145 (Apparatus U111 Unipress, Warszawa, Poland) coupled with a thermostat (Lauda Proline
146 RP 855 Low Temperature). The system consists of four high pressure vessels, working in
147 parallel, and each vessel has an internal volume of 8 mL. Each vessel is equipped with a
148 type K-thermocouple located at the bottom of the vessel, which allows recording the
149 temperature of the pressure transmitting medium (PTM). The PTM consisted of
150 propylene glycol which adiabatic heating is 5˚C per 100 MPa (Rasanavagam,
151 Balasubramaniam, Ting, Sizer, Bush, & Anderson, 2003). This system allows the
152 simultaneous application of high pressure (up to 600 MPa) and high temperature (up to
153 120˚C). Tubes (Cryogenic vial, Fisher Scientific, Canada) were filled with 2.7 mL of
154 untreated milk and pre-heated to a determined temperature considering that milk
155 increases its temperature by 3°C per 100 MPa (Buzrul, Alpas, Largeteau, Bozoglu, &
156 Demazeau, 2008). Once the pre-heated temperature was reached, the samples were
157 transferred to the high pressure vessels and pressurized at a rate of 6.89 MPa/s. During
158 the pressurization period, the temperatures of the sample and PTM increase due to
159 adiabatic heating (3 and 5˚C per 100 MPa, respectively). In addition, heat transfer also
160 occurs from the PTM to the sample (Rasanavagam, Balasubramaniam, Ting, Sizer, Bush,
161 & Anderson, 2003). With this experimental set up, it is assumed that the sample
162 temperature is similar to the PTM temperature as the sample volume in the polypropylene
163 test tube is small (~3 mL) compared to the PTM volume (~5 mL). At the end of the
164 holding time, the samples were decompressed and removed immediately from the high

pressure vessels and cooled down with ice to avoid further CLA degradation. For the different control treatments, samples of CLA-enriched milk in closed test tubes (Cryogenic vial, Fisher Scientific, Canada) were preheated in an oil bath at 42-100°C to simulate temperature conditions of PATS experiments. Then, the same samples were immersed in the thermostated oil bath for the PATS equipment kept at a constant temperature (60, 90 or 120°C). PATS-treated and control samples were kept at -18°C until further analysis. Additional sets of experiments adding catechin as an antioxidant to enhance CLA retention were conducted following the same experimental conditions. One gram of catechin (Sigma-Aldrich, Saint Louis, MO) per kg of untreated milk was added. CLA retention was expressed as a percentage of its initial amount before treatment. All experimental data were obtained in triplicate and all figures with error bars were made using Sigmaplot software V11 for windows (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Temperature and pressure history during PATS treatments

A typical PATS experimental run for CLA-enriched milk treated up to 14 min at 120°C and 600 MPa is shown in **Fig. 1**. The treatment time is the sum of the loading (t_l), compression (t_c), holding (t_h) and decompression (t_d) times. At the beginning of the treatment, a drop in the PTM was observed (**Fig. 1**, arrow (1)) when the preheated sample was inserted into the high pressure vessel. This temperature drop also indicates the start of the loading time, which is the time needed to insert the preheated sample, adjust the PTM volume and close the high pressure vessel. Thereafter, the sample is pressurized to the desired pressure. This period is known as the compression or pressure build-up time

(t_c). A drop in the transmitted fluid temperature is observed at the beginning of the compression time (**Fig. 1**, arrow 2) because the temperature of the pressurizing fluid that comes from the intensifier is lower than the fluid temperature in the vessel (120˚C). Due to adiabatic heating, the temperature of both sample and medium rises (Ting et al., 2002), creating a gradient temperature. The thermocouple located at the bottom of the high pressure vessel measures a drop of ~8˚C in the fluid temperature followed by a rise up to 128˚C. During t_c, the sample is submitted to non-isothermal and non-isobaric conditions over a relatively short period of time (105 s). In this study, a slow compression rate was used (~6.89 MPa/s) to allow better heat temperature distribution within the vessel as suggested earlier by De Heij, van Schepdael, van den Berg and Bartels (2002). The time at which the target temperature and pressure was reached is considered the start of the holding time. During this time, the sample is under near isothermal and isobaric conditions. Upon completion of the holding time, decompression is characterized by a drop in the medium temperature (**Fig. 1**, arrow (3). This period is usually short and indicates the end of the treatment.

Similar descriptions of PATS processes have been reported elsewhere (e.g. Balasubramaniam, Ting, Stewart, & Robbins, 2004; Ting et al., 2002; van den Ve, Courvoisier, & Matser, 2007; Mathys et al., 2009; Koutchma et al., 2010). A detailed description of PATS treatments is needed for the correct interpretation of experimental findings since differences in equipment size and process variables (PTM, compression rate, etc.) result in different pressure and temperature time profiles.

3.2. CLA retention in milk treated by PATS

The CLA retention of CLA-enriched milk treated at different pressures, temperatures, and treatment times is shown in **Fig. 2**. CLA retention for the control treatments decreased with an increase of temperature. After 14 min of PATS treatment, CLA retention values were 87.0 ± 1.4, 71.3 ± 1.7, and 59.5 ± 1.6 % at 60, 90, and 120°C, respectively. During thermal processing, CLA is lost through oxidation depending on the temperature and treatment time. Herzalla et al. (2005) determined CLA retention values of 94, 79 and 85 % after treatments of 2 min at 90˚C, 5 min at 95˚C (microwave heated) and 4 s at 140˚C (UHT), respectively. Another study showed that only 89% of CLA was retained in fortified milk treated for 16 s at 77˚C (Campbell et al., 2003). These authors as well as Yang et al. (2000) explained that the conjugated double bond system (e.g. CLA) is more susceptible to autooxidation than the non-conjugated double bond system (e.g. linolenic acid).

Two possible explanations can be used to interpret CLA losses in enriched milk (**Fig. 2a**). One possible reason is that AMF rich in CLA has a higher ratio of unsaturated to saturated fatty acids (1.36) than the ratio for non-enriched AMF (0.71) (Martinez-Monteagudo et al., 2012). This ratio, found in CLA-enriched AMF using the same milk, is the same obtained for the CLA-enriched milk. As previously reported (Martinez-Monteagudo et al., 2012), unsaturated fatty acids also oxidize faster than saturated fatty acids at low temperatures. Under non-isothermal conditions, the start temperature of oxidation for AMF rich in CLA, as measured by differential scanning calorimetry, shifts

234 to lower values as the ratio of unsaturated to saturated fatty acids increases (Martinez-
235 Monteagudo et al., 2012). The other possible reason is that CLA in enriched milk has
236 34.5 mg of CLA/g of fat, which is considerably higher than the content in non-enriched
237 milk (5 mg of CLA/g of fat). Oxidation of AMF rich in CLA follows a first-order
238 reaction as reported by Martinez-Monteagudo et al. (2012). These authors found that
239 AMF rich in CLA was more susceptible to oxidation due to its high content of conjugated
240 double bonds.
241
242 An initial drop in the CLA retention values was recorded between 0 and ~3 min,
243 which corresponded to the loading and pressure built-up period (**Figs. 2b-d**). In this
244 period, the samples were subjected to non-isothermal and non-isobaric conditions making
245 it difficult to interpret the initial drop. Oley et al. (2006) evaluated the initial drop in
246 buffer solution of ascorbic acid. They concluded that the dissolved oxygen is responsible
247 for the initial drop; however, more experimental evidence is needed for different reaction
248 systems.
249
250 The CLA was relatively stable up to 14 min of treatment at 100 MPa, regardless
251 of the temperature used (**Fig. 2b**). At 60, 90, and 120°C, the CLA was retained within the
252 range of 76 to 85 % (**Fig. 2b**). Similarly, up to 14 min of treatment at 60°C in the
253 pressure range of 350 to 600 MPa, 76 to 90 % of CLA was retained (**Figs. 2b-d**). Under
254 these combinations of temperatures and pressures (60°C with 100-600 MPa and 100 MPa
255 with 60-120°C), CLA losses were minor to moderate. Such combinations of pressure and
256 temperature allow the inactivation of vegetative microorganisms required for

257 pasteurization (Wilson, Dabrowski, Stringer, Moezelaar, & Brocklehurst, 2008). High
258 pressure homogenization of milk from cow, goat and ewe using 50-350 MPa and 20-
259 65°C was previously reported (Rodríguez-Alcalá, Harte, & Fontecha, 2009). The authors
260 observed no change of CLA content and isomers distribution at any tested condition.
261
262 The retention values of CLA rapidly decreased when samples were heated at 90
263 and 120°C and pressurized at 350 MPa (**Fig. 2c**). The same tendency was observed at 600
264 MPa (**Fig. 2d**). A dramatic degradation of CLA (~3 % of retention) was observed in
265 samples treated for 14 min at 600 MPa and 120°C. The oxidation of CLA was
266 accelerated by the combination of high pressure (600 MPa) and high temperature
267 (120°C). Pressure and temperature could affect three possible reaction steps, resulting in
268 an acceleration of CLA oxidation. First, pressure could promote homolysis and free-
269 radical reactions which are considered the most important mechanisms of oxidation
270 (O'Connor & O'Brien 2006). This situation would occur if their activation volumes are
271 negative or close to zero(Escobedo-Avellaneda et al., 2011; Segovia Bravo et al., 2011).
272 Isaacs (1981) reported activation volumes of thermal homolysis in the range of 0.3-13
273 cm^3/mol for different peroxide reaction systems. On the other hand, negative activation
274 volumes in the range of -5 to -15 cm^3/mol have been reported for the initiation and
275 propagation stages of free-radical reactions (Isaacs, 1981). Certainly, these investigations
276 showed that pressure can accelerate one or more oxidation stages; however, these
277 reported activation volumes cannot explain the dramatic decrease in the CLA retention
278 values (**Fig. 2d**).
279

A second effect could involve dissolved oxygen that becomes more reactive (ground oxygen) when pressure is applied. Okamoto (1992) reported that the lifetime of singlet oxygen increases significantly with pressures up to 400 MPa; however, to date, the role of oxygen under PATS conditions (90-120°C and 400-600 MPa) has not been studied. (Oey et al., 2006)

In the third effect, pressure induces changes in milk proteins, resulting in the release of complexed metals, such as Zn, Fe, and Cu that might catalyze oxidation reactions. This is observed during partial disruption of caseins through pressure treatment (Gaucheron et al., 1997; Schrader, Buchheim & Morr, 1997; Needs, Stenning, Gill, Ferragut, & Rich, 2000). Additional experimental evidence is needed to confirm the role of this mechanism since all previous studies were conducted in the low to mild temperature range (room temperature to 50^{o}C). In addition, the milk fat membrane globule (MFGM) contains high concentration of phosphatidylethanolamine (PE), which interacts with Cu^{2+}. During processing, the MFGM can be disrupted, releasing membrane phospholipids into the aqueous solution, thus increasing the proportion of PE in serum milk, depending on the severity of the treatment. The effect of PATS on mineral balance is currently under investigation in our laboratory.

3.3. CLA retention in anhydrous milk fat

An additional set of experiments were conducted in AMF rich in CLA (**Fig. 3**) in which the effect of metals catalyzing the reaction would be negligible. At 60 and 90°C and 0.1 MPa, CLA retention values decreased up to 14 min of treatment (86.9 ± 1.3 and

303 75.5 ± 1.1 %, respectively). These values were similar to those obtained at the same
304 pressure and temperature conditions for CLA-enriched milk (**Fig. 2a**); however, CLA
305 retention in AMF dropped initially from 100 to 59.5 ± 1.3 % at 120°C and 0.1 MPa,
306 remaining relatively stable up to 14 min of treatment (53.2 ± 1.3 %), with retention
307 values lower than those found for milk (59.3 ± 1.6 %). Fat in milk is emulsified and
308 protected from factors, such as enzymes, oxygen and metals, while the fat in AMF is
309 exposed to these factors, leading to a faster oxidation rate (Sharma, & Dalgleish, 1993).
310 Moreover, oxidation in milk is a result of complex interactions between pro- and
311 antioxidant compounds as reviewed by Lindmark-Mansson, & Akesson (2000).
312
313 At 100 and 350 MPa, similar values of CLA were obtained for AMF as compared
314 with those obtained for milk (**Figs. 2b and 2c**); however, there is a considerable
315 difference in CLA retention values obtained with AMF and milk at 600 MPa (**Figs. 2d**
316 and **3c**). Up to 14 min of treatment and 600 MPa, the CLA retention values in AMF were
317 91.9 ± 2.8, 65.6 ± 2.4, and 40.2 ± 2.0 % at 60, 90 and 120°C, respectively, while the
318 corresponding CLA retention values in milk were 76.2 ± 3.1, 39.8 ± 2.1, and 3.5 ± 2.0 %
319 at the same temperature and pressure conditions. In AMF where the presence of metals is
320 minimal and therefore their catalytic effect can be neglected, the retention values of CLA
321 (40.2 ± 2.0 %) were considerably higher than those obtained in milk up to 14 min of
322 treatment (3.5 ± 2.0 %).
323
324 CLA retention values were also obtained for AMF treated at 0.1-600 MPa and
325 60-120°C (**Figs. 3a** and **3b**). At the beginning of the treatment, there is an initial drop in

326 the CLA values from 100 to 59.5 ± 1.3 % at 0.1 MPa. This might be an indication that the
327 first stage of oxidation (radical formation by homolysis) occurred. This reaction involves
328 a covalent bond breaking, which would be delayed by pressure. Earlier, Tausher (1995)
329 slowed down the autoxidation of linolenic acid by using a pressure of 600 MPa. Using
330 pressure treatments of 100-600 MPa in this study, the initial drop in the CLA values was
331 not observed at any evaluated temperature, suggesting that pressure delays the oxidation
332 of CLA; however, at 600 MPa and 120°C, the retained CLA in AMF was lower than at
333 0.1 MPa and 120°C (40.24 ± 2.10 and 59.25 ± 1.55 %, respectively), suggesting that
334 propagation of oxidation was accelerated by pressure. Propagation is a free radical
335 reaction that is expected to be accelerated by pressure as suggested by Isaacs (1981).
336
337 **3.4. Formation of lipid hydroperoxides**
338 CLA is not stable upon processing and is likely oxidized. During oxidation, free
339 radicals are formed through thermolysis and due to the presence of enzymes and active
340 oxygen species. These radicals react with molecular oxygen to form products such as
341 hydroperoxides. These compounds are unstable and further react through free radical
342 mechanisms to form secondary products that propagate the oxidation. Thus, the detection
343 and quantification of hydroperoxides in PATS-treated samples can indicate an early stage
344 of lipid oxidation. The hydroperoxide content in enriched and non-enriched milk treated
345 at 600 MPa and 90 and 120°C is shown in **Fig. 4**. Interestingly, the 14 min treatment at
346 120°C and 600 MPa produced a hydroperoxide content higher in non-enriched milk than
347 in enriched milk (6.5 ± 0.1 and 5.26 ± 0.21 mmol/mL, respectively). The relatively low
348 content of hydroperoxides in enriched milk is not surprising since conjugated double

bond systems have more than one type of primary oxidation product and more than one oxidation pathway, as reported elsewhere (Brimberg, & Kamal-Eldin, 2003; Hamalainen, Sundberg, Makkinen, Hase, & Hopia, 2001). A kinetic analysis on autoxidation of methyl-conjugated linoleate showed that monomeric and cyclic peroxides are the major primary oxidation products rather than hydroperoxides (Hamalainen et al., 2001). Consequently, addition by Diels Alder-type reaction was suggested as a reaction mechanism. In general, Diels-Alder reactions are strongly accelerated by pressure with activation volumes in the range of -30 to -40 cm^3/mol (Isaacs, 1981). This is because cyclic compounds have smaller partial molar volumes than acyclic analogous (Walling, & Waits, 1967).

Another reason for the relative low content of hydroperoxides is that CLA can act as an antioxidant capturing those free radicals responsible for lipid oxidation. This antioxidant behaviour has been demonstrated by Fagali, & Catala (2008), who induced lipid oxidation in fish oil by adding tert-butyl hydroperoxide and found that CLA effectively reduces lipid peroxidation as measured by chemiluminescence. Additionally, these authors also reported antiradical or scavenging ability of CLA isomers measured by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) technique. Antioxidant and antiradical ability depend on CLA concentration. Martinez-Monteagudo & Saldaña (2011) demonstrated that CLA preserves its antiradical ability as measured by DPPH technique within 14 min of treatment at 600 MPa and 120°C. Thus, after pressure treatment, CLA can still donate hydrogen to form a CLA-free radical that further reacts to inhibit hydroperoxides formation (Martinez-Monteagudo & Saldaña, 2011).

3.5. Effect of catechin on CLA retention in milk and anhydrous milk fat

CLA is lost to a greater extent in milk as compared to AMF as shown in **Figs. 2** and **3**. To enhance the retention of CLA, catechin, a potent antioxidant capable of capturing free radicals and quenching metals (Yang et al., 2000), was added. Catechin effectively retained CLA in milk and in AMF regardless of the pressure and temperature used (data not shown). For instance, 90.2 ± 3.5 and 90.5 ± 2.0 % of CLA were retained in milk and in AMF at 600 MPa, 120˚C and up to 14 min of treatment (**Fig. 5**). Further research is needed to evaluate the effectiveness of the concentration of the antioxidant and the mechanism by which this antioxidant captures free radicals or reacts with the dissolved oxygen to retard the free radical reactions under PATS conditions.

4. Conclusions

For the first time, the effect of pressure-assisted thermal sterilization conditions on CLA retention in enriched milk was studied. After 14 min of treatment at 100 MPa, at least 80% of CLA was retained, regardless of temperature used. CLA was not stable up to 14 min of treatment at 600 MPa and 120°C with a retention value of approximately 3 %. Under the same PATS conditions, CLA was lost to a greater extent in enriched milk than in enriched AMF. Possibly, CLA is lost through an oxidation reaction that is catalyzed by free metal ions released when applying pressure. This might limit the applicability of this technology in preserving the biological activity of CLA in milk. However, the addition of catechin at 1g/kg of milk effectively enhanced CLA retention (> 90%) at any PATS

condition in both milk and AMF. Further research is needed to unveil the reaction mechanisms of antioxidants at PATS conditions.

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Figure 1:

Temperature and pressure history during pressure-assisted thermal sterilization treatments. Enriched CLA milk was treated for 14 min at 120˚C and 600 MPa (t_l, t_c, t_h, and t_d – loading, compression, holding and decompression time, respectively, at a compression rate of 6.89 MPa/s).

Figure 2:

Retention of conjugated linoleic acid in milk treated at 0.1 (a), 100 (b), 350 (c) and 600 MPa (d) and temperatures of 60, 90 and 120˚C.

Figure 3:

Retention of conjugated linoleic acid in anhydrous milk fat treated at 0.1 (a), 100 (b), 350 (c) and 600 MPa (d) and temperatures of 60, 90 and 120˚C.

Figure 4:

Formation of lipid hydroperoxides in enriched and non-enriched milk treated up to 14 min at 90 and 120°C and 600 MPa.

Figure 5:

Retention of conjugated linoleic acid in milk and in AMF at 600 MPa, 120°C and up to 14 min of treatment.

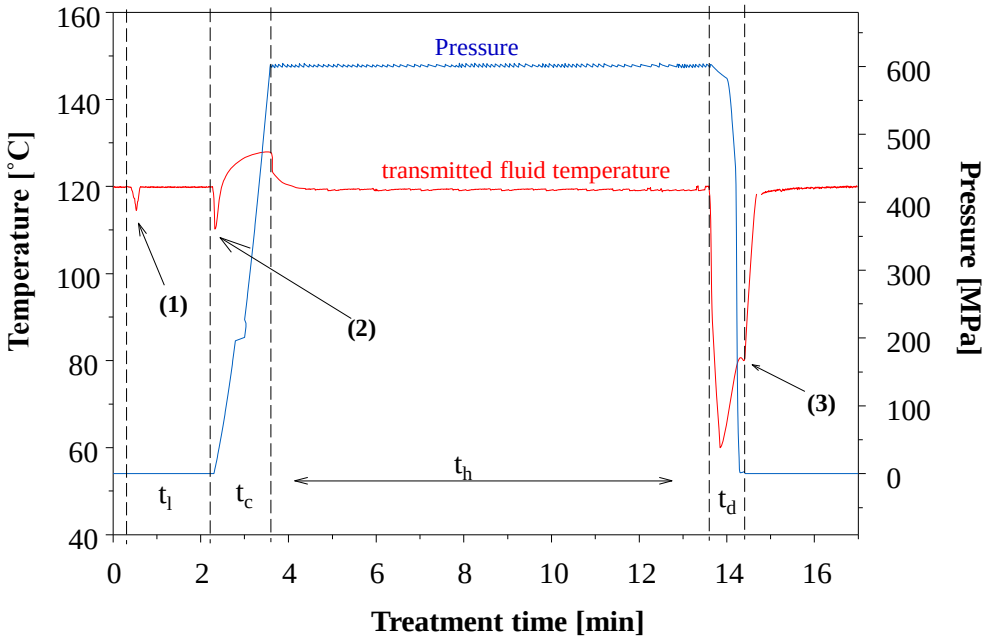
Pressure
transmitted fluid temperature
(1)
(2)
(3)
t_l
t_c
t_h
t_d
Temperature [°C]
Pressure [MPa]
Treatment time [min]
160
140
120
100
80
60
40
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16

Figure 1

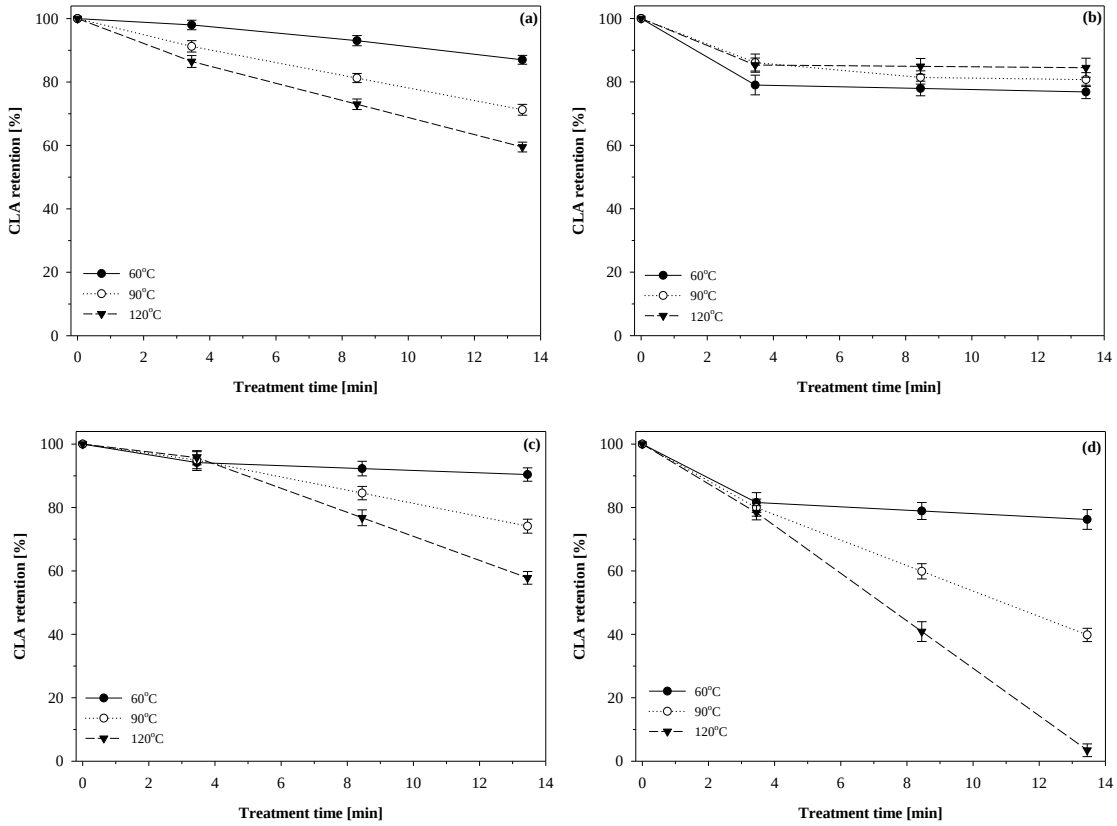

Figure 2

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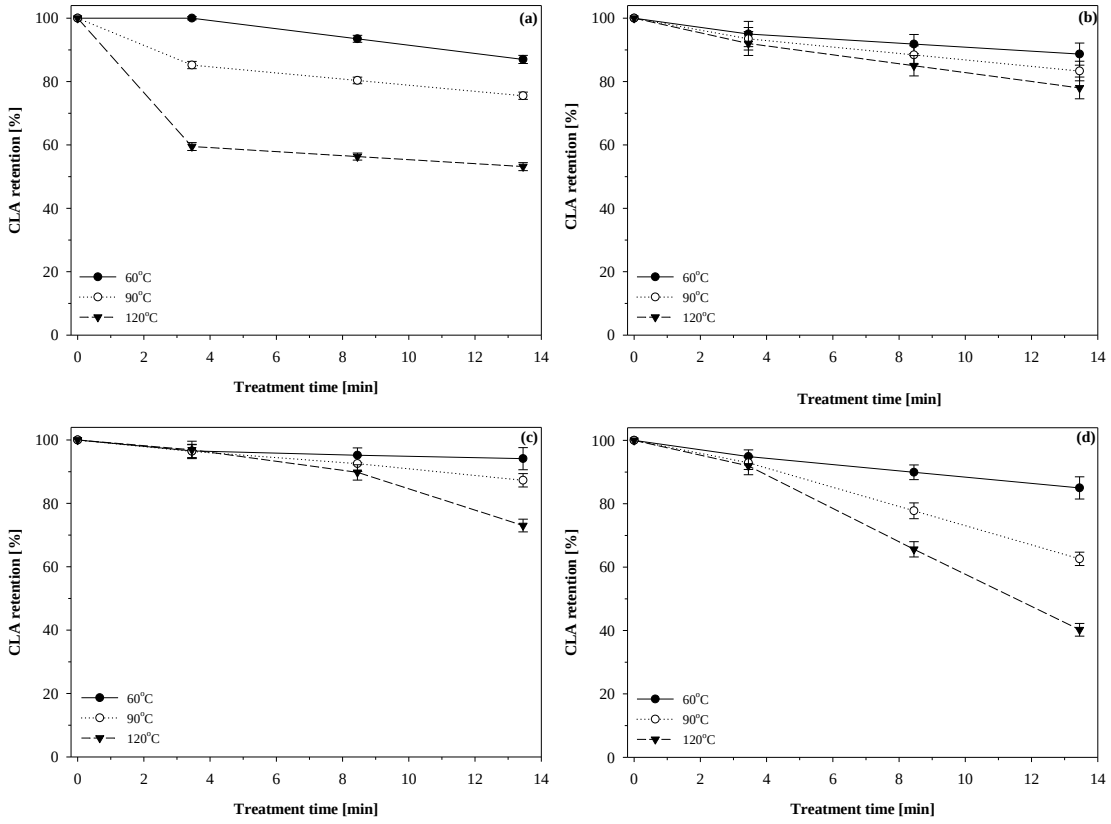

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611 **Figure 3:**

612

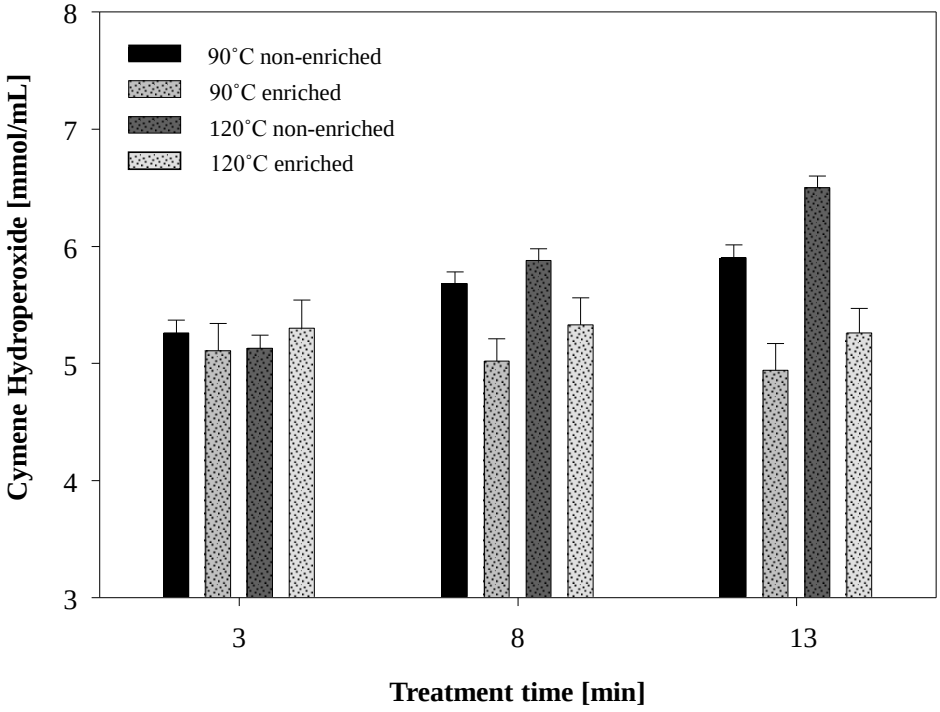
90˚C non-enriched
90˚C enriched
120˚C non-enriched
120˚C enriched
Cymene Hydroperoxide [mmol/mL]
8
7
6
5
4
3
3
8
13
Treatment time [min]

Figure 4:

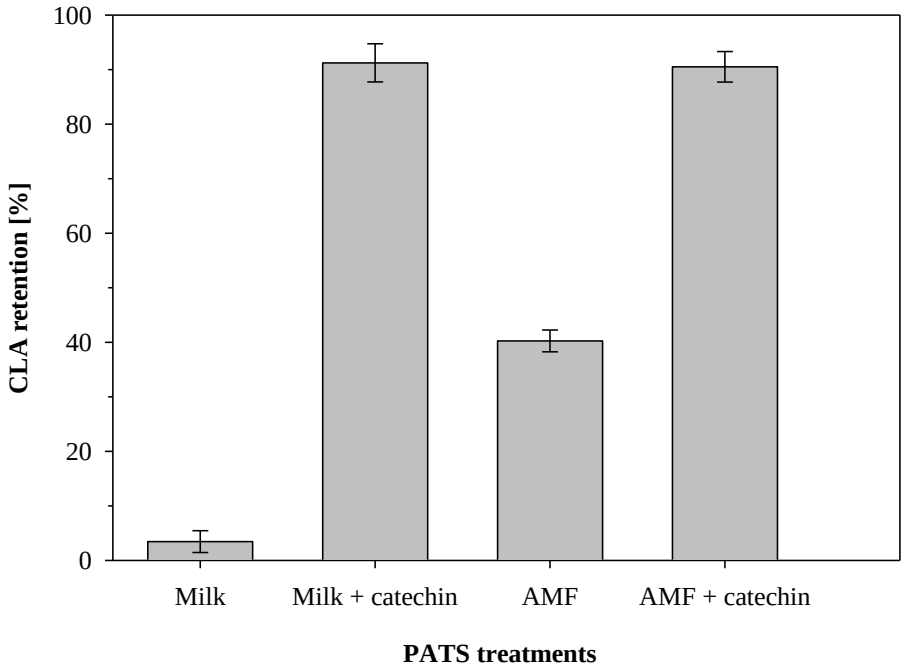

Figure 5:

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