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Combined pressure–temperature effects on the chemical marker (4-hydroxy-5-methyl- 3(2H)-furanone) formation in whey protein gels

Rockendra Gupta [a], Galina Mikhaylenko [c], V.M. Balasubramaniam [a,b,*], Juming Tang [c]

[a] Department of Food Science and Technology, The Ohio State University, Columbus, OH 43210, USA
[b] Department of Food Agricultural and Biological Engineering, The Ohio State University, Columbus, OH 43210, USA
[c] Department of Biological Systems Engineering, Washington State University, Pullman, WA, USA

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ABSTRACT

Chemical markers, such as furanone, are intrinsically formed in foods at elevated process temperatures, and have been successfully used as indirect indicators of heating patterns in advanced thermal processes such as aseptic processing, microwave sterilization and ohmic heating. However, very limited information is available on suitability of these chemical markers during combined pressure–heat treatment. The present study was conducted on the formation and stability of chemical marker M-2 (4-hydroxy-5-methyl-3(2H) furanone, a by-product of Maillard reaction) as a function of pressure, temperature and pH. Whey protein gels (containing 1g ribose/100g gel mix) at pH 6.1 and 8.3 were subjected to pressure assisted thermal processing (PATP; 350 and 700 MPa, 105 °C), high pressure processing (HPP; 350 and 700 MPa, 30 °C) and thermal processing (TP; 0.1 MPa, 105 °C) for different holding times. Unprocessed gel was used as control. The marker yield was quantified using HPLC. The initial concentrations of M-2 in the gels were 9.17 and 6.1 mg/100 g at pH 6.1 and 8.3, respectively. As expected, heat treatment at 105 °C, 0.1 MPa increased M-2 concentration. The marker yield increased with increase in holding time, following a first order kinetics and decreased with increasing pH. Pressure treatments from 350 to 700 MPa at 30 °C reduced the chemical marker formation for both pH values investigated. Marker formation during combined pressure-temperature (105 °C, 350 and 700 MPa) was influenced by both heat (which favored the marker formation) and pressure (which hindered marker formation). The net final concentration of the marker formed during PATP was higher than HPP, but lower than thermal treatments. This study suggests that 4-hydroxy, 5-methyl, 3(2H) furanone may not be a suitable marker for evaluating pressure–heat uniformity during PATP.

1. Introduction

Pressure-assisted thermal processing (PATP) offers new opportunities to the food industry for processing high quality low-acid, shelf-stable foods. Although shelf-stable, low-acid foods processed by this technology are not current commercially available, the technology can be used for processing heat-sensitive products such as mashed potatoes, dinner kits, meats and sauces, soups, egg products, coffee, and tea (Balasubramaniam & Farkas, 2008; Juliano et al., 2006). During a typical PATP process, the food is subjected to a combination of elevated pressures (500–900 MPa) and moderate heat (90–121 °C) for a short time (≤15 min). One of the unique advantages of PATP is its ability to provide a rapid increase in the temperature of treated food samples. Rapid compression heating and subsequent expansion cooling on decompression help to reduce the severity of thermal effects encountered with conventional processing techniques (Rajan, Ahn, Balasubramaniam, & Yousef, 2006).

The sample temperature increase when exposed to high pressure, also known as heat of compression, is well documented in the literature (Delgado, Baars, Kowalczyk, Benning, & Kitsubun, 2007; Patazca, Koutchma, & Balasubramaniam, 2007; Rasanayagam et al., 2003; Torres, Sanz, Otero, Perez-Lamela, & Saldana, 2009). This increase in temperature is influenced by food composition, product initial temperature and target pressure. As a result, temperature gradient may exist within the pressure vessel, possibly due to the differences in the thermal properties such as specific heat capacity, thermal conductivity and physicochemical properties such as composition, density, etc. (Ramaswamy, Balasubramaniam, & Sastry, 2005. pp. 1–6). Previous attempts to study the temperature distribution profile within the high pressure chamber involve use of

* Corresponding author. Department of Food Science and Technology, The Ohio State University, Columbus, OH 43210. Tel.: +1 614 292 1732; fax: +1 614 292 0218.
E-mail address: balasubramaniam.1@osu.edu (V.M. Balasubramaniam).

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thermo-fluid dynamic based mathematical models (Carroll, Chen, & Fletcher, 2003; Chen, Zhu, Ramaswamy, Marcotte, & Le Bail, 2007; Hartmann & Delgado, 2005; Hartmann et al., 2004) and enzyme/protein based time-temperature-pressure indicators (Gogou, Katapodis, & Taoukis, 2010; Grauwet, Plancken, Vervoort, Hendrickx, & Van Loey, 2010; Rauh, Baars, & Delgado, 2009; Van der Plancken, Grauwet, Oey, Van Loey, & Hendrickx, 2008). Most enzyme based time-temperature-pressure indicators have been studied at high pressure processing (HPP) conditions utilizing modest process temperatures (<70 °C).

Development of biochemical indicators to monitor thermal process non-uniformities within a pressure chamber during PATP could help the industry to ensure product safety and optimize the process. Kim and Taub (1993) suggested that certain chemical compounds, such as Maillard reaction products, formed in the food during thermal processing could be used as indicators of heating patterns. Three biochemical markers have been commonly identified in foods, viz. 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (referred to as M-1), 4-hydroxy-5-methyl-3(2H)-furanone (M-2) and 5-hydroxymethylfurfural (M-3) (Kim & Taub, 1993). These markers are the degradation products of Maillard reaction between carbonyl group of a reducing sugar and amine group of a suitable reactive amino acid (Lau et al., 2003; Wang, Lau, Tang, & Mao et al. 2004). Number of earlier studies (Kim et al., 1996; Ramaswamy, Awuah, Kim, & Choi, 1996) utilized these markers for investigating temperature distribution during ohmic and aseptic processing. The relatively fast reaction rates for the formation of marker M-2 in protein rich substrates at temperature beyond 100 °C make this marker particularly useful in studying heat distribution during short-time sterilization processes (Lau et al., 2003; Pandit, Tang, Liu, & Mikhaylenko, 2007; Pandit, Tang, Mikhaylenko, & Liu, 2006). Also, M-2 marker yield can be positively correlated with thermal lethality and, thus, can be effectively used to locate colds spots in packaged foods during microwave sterilization processes (Pandit, Tang, Liu, & Pitts, 2007).

The objective of this study was to evaluate the feasibility of using the chemical markers to investigate temperature induced process non-uniformities during combined pressure–heat treatment.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI), 93.2 g/100g (Bipro) (wet basis), and whey protein concentrate (WPC), 78.4 g/100g (wet basis), were purchased from Davisco foods international, Eden Prairie, MN, USA. WPC had 8.41g lactose/100g WPC (wet basis). D-ribose (≥99 g/100g) and 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (purum ≥99 g/100 g) (M-2 chemical marker standard) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Non-iodized food grade salt (Morton International, Chicago, IL, USA) was purchased from a local grocery store. Sodium acetate (anhydrous) and potassium bicarbonate (99.5–101.5 g/100g USP) were purchased from Fisher scientific, Pittsburgh, PA, USA.

2.2. Buffer solutions

10g/100 ml acetate buffer (pH 7.0) and 5 g/100 ml bicarbonate buffer (pH 10.0) were prepared by dissolving respective amounts of sodium acetate and potassium bicarbonate in water.

2.3. Preparation of whey protein gels

Whey protein gels were prepared by modifying the formulation suggested by Lau et al. (2003). The formulation was modified (by optimizing the concentrations of whey protein concentrate, isolate and water) to minimize syneresis of whey protein gels under high pressure and thus prevent the migration of M-2 marker. 200 g batches of whey protein gel consisted of 24 g whey protein concentrate/100g gel mix, 11g whey protein isolate/100 g gel mix, 1 g D-ribose/100 g gel mix, 0.8 g salt/100 g gel mix and 63.2 g de-ionized water/100 g gel mix. Lactose from WPC amounted to ~2 g/100 g gel mix. The total amount of protein contributed by WPC and WPI was ~29 g/100 g gel mix. Briefly, respective quantities of D-ribose and salt were dissolved in water and mixed with weighed amounts of WPC and WPI in a lab blender (Oster 10 speed blender, Boca Raton, FL, USA) for 1 min to obtain a paste like consistency. The natural pH of the paste was 6.1. Appropriate amounts of sodium acetate and potassium bicarbonate buffer solutions were added to prepare paste with a pH of 8.3. The samples were stored overnight under refrigerated conditions to ensure complete protein hydration. 200g hydrated paste was poured in rectangular Nalgene bottle and the bottle were covered with aluminum foils to prevent evaporation of water during gel formation. Gel was formed by placing the Nalgene bottles containing the hydrated paste in a water bath (Isotemp 128, Fisher Scientific, Pittsburgh, PA, USA) maintained at 80 °C for 40 min. The whey protein gel was immediately cooled in an ice–water mixture and refrigerated at 4 °C till further processing and analysis. Earlier studies have shown that heating at 80 °C for 40 min causes negligible browning in whey protein gels (Lau et al., 2003), the gels containing D-ribose show prominent browning at temperatures only above 100 °C (Pandit, Tang, Liu, & Mikhaylenko, 2007). The gels contained 9.17 mgM-2 marker/100 g whey protein gel.

2.4. Processing the whey protein gels

Uniform 7 cm × 2 cm × 0.1 cm sections were cut from the whey protein gel blocks and immediately vacuum packaged (Spiromac vacuum sealer, model 450 T, Québec, Canada) in polypropylene pouches (76.2 μm) Deli, NS1D30-155215, Thomson equipment and supply (Cincinnati, OH, USA). Vacuum packaging did not have any observable effect on the physical characteristics of the gel. The packaged samples were subjected various pressure–heat combinations as outlined below.

2.5. High-pressure kinetic tester

A high-pressure kinetic tester (pressure test unit PT-1, Avure Technology Inc., Kent, WA, USA) was used to process the gel samples. A 54-ml stainless steel (SS-316) pressure chamber was immersed in a temperature-controlled bath to maintain the desired process conditions (30 °C for HPP and 105 °C for PATP). Propylene glycol (57-55-6, Avatar Corporation, University Park, IL, USA) was used as the pressure transmitting medium as well as heating medium in the temperature-controlled bath. The desired pressure was generated at the rate of 18.42 MPa/s using an intensifier (M-340 A, Flow International, Kent, WA, USA) connected to a hydraulic pump (model PO45/45-OGPM-120, Interface Devices, Milford, CT, USA). The depressurization time was approximately 2 s. The pressure holding times provided in Table 1 do not include pressurization or depressurization times. More details about the equipment are described elsewhere (Rajan et al., 2006).

2.6. High pressure processing

Whey protein gel samples (~3.5 g each) vacuum sealed in pouches were compressed to 350 or 700 MPa and held for 0, 5, 10 and 20 min at 30 °C in the high pressure PT-1 kinetic tester. Before HPP treatment, the samples were preconditioned in ice–water mixture for 10 min and placed inside a 10-ml polypropylene

Table 1
Temperature histories at different stages of processing during high pressure processing (HPP; 350 and 700 MPa at 30 °C) and pressure-assisted thermal processing (PATP; 350 and 700 MPa at 105 °C) of whey protein gel samples.

Treatment	Processing Pressure (MPa)	Processing time (min)	Temperature at different stages during processing (°C)					Time required at different stages of preprocessing (s)	
			Preprocess (T_1)	Immediately before pressurization (T_2)	Immediately after pressurization (T_3)	Pressure holding (T_3-T_4)	Depressurization (T_5)	Preprocess (t_1)	Pressure come up time (t_2)
HPP 30 °C	350	0	14.0 ± 1	17.3 ± 0.6	28.2 ± 1.1	30.0 ± 0.4	19.1 ± 0.8	618 ± 5	35 ± 2
		5	14.0 ± 1	17.3 ± 0.6	28.2 ± 1.1	30.3 ± 0.2	19.1 ± 0.8	618 ± 5	35 ± 2
		10	14.0 ± 1	17.3 ± 0.6	28.2 ± 1.1	30.1 ± 0.4	19.1 ± 0.8	618 ± 5	35 ± 2
		20	14.0 ± 1	17.3 ± 0.6	28.2 ± 1.1	30.3 ± 0.2	19.1 ± 0.8	618 ± 5	35 ± 2
	700	0	1.0 ± 1	7.0 ± 0.5	28.4 ± 0.7	30.6 ± 0.6	11.6 ± 0.9	618 ± 5	35 ± 2
		5	1.0 ± 1	7.0 ± 0.5	28.4 ± 0.7	30.8 ± 0.4	11.6 ± 0.9	618 ± 5	35 ± 2
		10	1.0 ± 1	7.0 ± 0.5	28.4 ± 0.7	30.4 ± 0.7	11.6 ± 0.9	618 ± 5	35 ± 2
		20	1.0 ± 1	7.0 ± 0.5	28.4 ± 0.7	30.1 ± 0.3	11.6 ± 0.9	618 ± 5	35 ± 2
PATP 105 °C	350	0	84 ± 1	87.8 ± 0.9	103.4 ± 0.5	105.1 ± 0.5	89.1 ± 1.1	326 ± 7	35 ± 2
		5	84 ± 1	87.8 ± 0.9	103.4 ± 0.5	104.9 ± 0.6	89.1 ± 1.1	326 ± 7	35 ± 2
		10	84 ± 1	87.8 ± 0.9	103.4 ± 0.5	105.2 ± 0.5	89.1 ± 1.1	326 ± 7	35 ± 2
		20	84 ± 1	87.8 ± 0.9	103.4 ± 0.5	105.3 ± 0.4	89.1 ± 1.1	326 ± 7	35 ± 2
	700	0	57 ± 1	69.5 ± 0.9	103.9 ± 0.7	105.5 ± 0.4	77.1 ± 1.2	326 ± 7	35 ± 2
		5	57 ± 1	69.5 ± 0.9	103.9 ± 0.7	105.2 ± 0.4	77.1 ± 1.2	326 ± 7	35 ± 2
		10	57 ± 1	69.5 ± 0.9	103.9 ± 0.7	104.9 ± 0.6	77.1 ± 1.2	326 ± 7	35 ± 2
		20	57 ± 1	69.5 ± 0.9	103.9 ± 0.7	104.7 ± 0.3	77.1 ± 1.2	326 ± 7	35 ± 2

syringe (model 309604, Difo, Becton Dickinson, Franklin Lakes, NJ, USA), which served as the sample holder. After loading the pouch inside the sample holder, it was filled with approximately 6 ml of chilled water (from the ice–water mixture) to ensure that immediate vicinity of the sample pouch had similar temperature and heat of compression characteristics as that of the whey protein gel. To minimize heat exchange with the surrounding glycol in bath, the sample holder was wrapped with two layers of insulating material (Sports Tape, CVS pharmacy Inc., Woonsocket, RI, USA) (Nguyen, Rastogi, & Balasubramaniam, 2007). The initial temperatures of the samples were determined by using the following equation (Nguyen et al., 2007; Rasanayagam et al., 2003) and by performing preliminary experiments.

$$T_3 = T_2 + \frac{\sum_i (CH_i * M_i)}{1}\left(\frac{\Delta P}{100}\right) + \Delta T_H \quad (1)$$

where, T_3 is the target temperature, T_2 is the initial sample temperature just before the commencement of pressurization, CH_i is the heat-of-compression value of component i of the sample (defined as temperature increase per 100 MPa during sample pressurization), M_i is the mass fraction of component i in the sample, ΔP is the process pressure and ΔT_H is the temperature gain (lost) by the test sample from (to) the surrounding glycol bath. Sample temperature history at various stages of high pressure treatment is given in Table 1. After processing, the samples were immediately withdrawn and stored at 4 °C until analyzed.

2.7. Pressure assisted thermal processing

Whey protein gel samples were also pressure (350 and 700 MPa) treated under elevated heat (105 °C) conditions for 0, 5, 10 and 20 min using high pressure PT-1 kinetic tester described earlier. The samples and the sample holder (10-ml polypropylene syringe) were preconditioned in a hot water bath (Isotemp 128, Fisher Scientific, Pittsburgh, PA, USA) for 5 min. The water bath was maintained at respective predetermined temperatures for each of the pressures (see Table 1). The sample pouch was then inserted in the syringe and remainder of the syringe was filled with warm water and immediately inserted in the pressure chamber for pressurization. The temperature history of samples at various stages of PATP treatments is given in Table 1. After processing, the samples were immediately cooled in ice–water mixture and subsequently stored at 4 °C until analyzed.

2.8. Thermal processing

Preheated glycol at 105 °C in the temperature control bath of the high pressure vessel was used for thermal processing (105 °C for 0, 5, 10 and 20 min) of whey protein gel samples. Whey protein gel pouches (discussed in previous sections) were immersed in hot propylene glycol (105 °C) and held for 0, 5, 10 and 20 min after which they were immediately cooled in ice–water mixture and refrigerated at 4 °C until analyzed.

2.9. High pressure liquid chromatography (HPLC)

Chemical marker (M-2) yields were quantified using HPLC. HP 1050 system (Hewett Packard, Plainsboro, NJ, USA) equipped with a Waters photodiode array detector (Waters Corp, Milford, MA, USA) and a solvent delivery system was used. 1.2 g sample was ground in a mortar pestle with 10 ml 10 mmol/L H_2SO_4. The homogenous paste was centrifuged (527 × g) for 10 min and the supernatant was filtered through 0.45 μm nylon membrane filters in HPLC vials. The filtrate was injected into an HPLC fast acid analysis column (Bio-RAD, Hercules, CA, USA) using automatic injection system (HP 1050, Hewlett Packard Co., Plainsboro, NJ, USA). 10 mmol/L H_2SO_4 at a flow rate of 1 ml/min was used as the mobile phase. Absorbance of the eluting compounds was measured at 285 nm (Kim & Taub, 1993).

To quantify and characterize M-2 (4-Hydroxy-5-methyl-3(2H)-furanone) concentration in control and processed whey protein gels, a standard M-2 curve was generated using 4-hydroxy-2,5-dimethyl-3(2H)-furanone standard obtained from Sigma Aldrich (St. Louis, MO, USA). Concentration of the M-2 marker in processed and control samples was expressed as mg/100 g whey protein gel.

2.10. Kinetics of M-2 formation

Formation of M-2 in whey protein gels containing 1g ribose/100g gel mix is given by eqn. (2) (Lau et al., 2003)

$$\frac{dC}{dt} = k(C_\infty - C)^n \quad (2)$$

Where C_∞ is the concentration at saturation and C is the concentration at time *t*. *k* is the reaction rate constant and *n* is the reaction order.

2.11. Statistical data analysis

Data was analyzed with Minitab software, version 14.1 (Minitab, State College, PA, USA). The influence of treatment type (HPP, PATP, or TP), treatment time, gel pH as well as their interaction on the formation chemical marker M-2 in whey protein gels was analyzed. Pairwise comparisons for the means of treatment variables (factors) were evaluated with Tukey's test at 5% significance level ($P < 0.05$).

3. Results and discussion

3.1. Formation of M-2 (4-hydroxy-5-methyl-3(2H)-furanone) under combined pressure–heat treatment conditions

A typical chromatogram of standard 4-Hydroxy-5-methyl-3(2H)-furanone at 285 nm is shown in Fig. 1. The marker compound eluted between 5 and 8 min.

Chemical marker M-2 (4-Hydroxy-5-methyl-3(2H)-furanone) formation in whey protein gels (containing 1g ribose/100g gel mix) as a function of pressure (350 and 700 MPa), temperature (30 and 105 °C) and holding time (0, 5, 10 and 20 min) is shown in Fig. 2 corresponding to pH 6.1 and Fig. 3 corresponding to pH 8.3.

At pH 6.1, the formation of M-2 marker in whey protein gels during thermal processing (0.1 MPa and 105 °C) increased with holding time (Fig. 2). However, whey protein gels treated with combined pressure-temperature combinations showed a distinct inhibitory/degradation effect on M-2 formation. The magnitude of this inhibitory and/or degradation effect increased as the pressure was increased from 350 to 700 MPa (see Fig. 2). Under combined pressure-temperature treatments, increase in temperature from 30 to 100 °C increased the M-2 marker yield. Within the experimental conditions of the study, increase in time did not show a significant increase in the marker formation for samples processed at 700 MPa, 105 °C and 350–700 MPa at 30 °C. Thus, maximum M-2 marker yield was obtained in thermally processed gels held at 0.1 MPa, 105 °C for 20 min, whereas the minimum M-2 marker was formed in whey protein gel samples processed at 700 MPa and 30 °C.

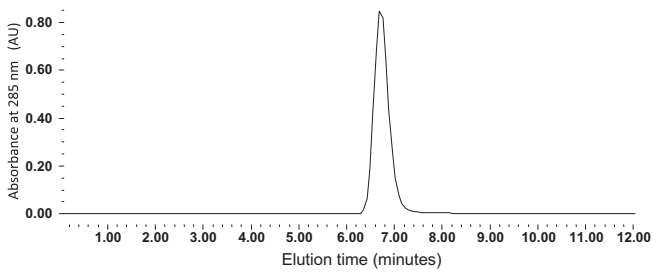

Fig. 1. HPLC Chromatogram of chemical marker M-2 (4-hydroxy-5-methyl-3(2H)-furanone) standard extracted at 285 nm.

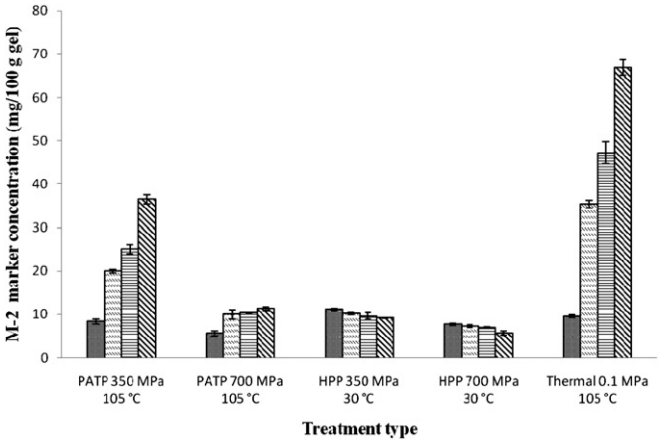

Fig. 2. Concentration of M-2 marker in whey protein gels, pH 6.1, subjected to combinations of two process temperatures (30 and 105 °C), two pressures (350 and 700 MPa) and four holding times (■ 0, ◹ 5, □ 10 and ◹ 20 min). Unprocessed gel contained 9.17 mg M-2 marker/100 g gel. Bars represents mean ± SD, $n = 3$.

3.2. Kinetics constants for marker formation during various pressure–heat treatments

The marker formation during thermal processing followed a first order kinetics. This is in agreement with that reported by other researchers for thermal processing studies (Lau et al., 2003). The rate constants of marker M-2 formation under different processing conditions are summarized in Table 2. The rate constant in thermally processed gel samples (0.1 MPa, 105 °C) was 0.077 min^{-1}, whereas application of pressure (350 and 700 MPa) at 105 °C inhibited the marker formation. The rate constants for marker formation under 350 and 700 MPa at 105 °C were 0.023 and 0.0033 min^{-1} respectively. Treatment of gel samples at 350 and 700 MPa at low temperature (30 °C) showed negative rate constants (Table 2 −0.0013, −0.0014 min^{-1} respectively) for the M-2 marker formation, thus confirming degradation/inhibition effects of pressure on the formation of M-2.

At pH 8.3 the marker yield was significantly lower ($p < 0.05$) than that at pH 6.1 regardless of the temperature and pressure used (Fig. 3). Although the formation of M-2 followed first order reaction kinetics at 0.1 MPa and 105 °C and M-2 concentration increased with holding time, two consecutive reactions viz. formation and decay were seen to occur during 350 and 700 MPa pressure

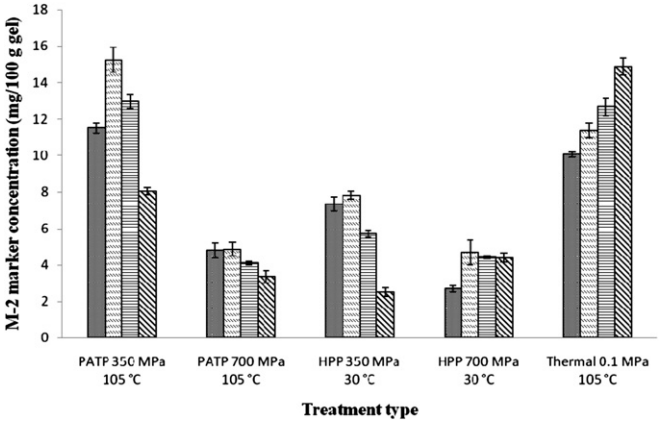

Fig. 3. Concentration of M-2 marker in whey protein gels, pH 8.3, subjected to combinations of two process temperatures (30 and 105 °C), two pressures (350 and 700 MPa) and four holding times (■ 0, ◹ 5, □ 10 and ◹ 20 min). Unprocessed gel contained 6.1 mg M-2 marker/100 g gel. Bars represents mean ± SD, $n = 3$.

Table 2
Rate constants of marker M-2 formation/inhibition/degradation under different processing conditions at gel pH 6.1 and pH 8.3.

Processing conditions			Rate constant ($k\ min^{-1}$)[a]	Correlation coefficient (R^2)
pH	Temperature (°C)	Pressure (MPa)		
6.1	105	350	0.0232	0.99
		700	0.0033	0.69
		0.1	0.0772[a]	0.99
	30	350	−0.0013[a]	0.87
		700	−0.0014[a]	0.97
8.3	105	350	−0.04	0.3[b]
		700	−0.022	0.89
		0.1	0.056	0.99
	30	350	−0.006	0.92
		700	0.004	0.34[b]

[a] Negative sign indicates degradation/inhibition of marker M-2 under combined pressure-temperature conditions. Whereas temperature favored the formation of M-2, pressure had a degradation/inhibition effect on M-2.
[b] At pH 8.3, the formation/degradation/inhibition of M-2 marker did not follow first order kinetics. Hence the lower values of correlation coefficients.

treatment. Under combined pressure-temperature conditions (350 and 700 MPa, 30 and 105 °C) an increase in concentration of M-2 was seen during the first 5 min of processing after which a decrease was observed. This observation is similar to that reported at pH 7.0 by Bristow and Isaacs (1999). The differences in concentration and time required to reach the maximum could be attributed to differences in pH values, differences in reaction systems and processing conditions used. At either pH (viz. 6.1 and 8.3), the extent of browning in the gel samples could not be directly related to the M-2 marker formation. The significance of independent variables (time, temperature, pH and pressure) and their interactions in the formation of M-2 marker is shown in Table 3. It is interesting to note that each of the variables contributed significantly toward the marker yield ($p < 0.05$).

The effects of pressure, temperature and pH on the formation of M-2 (a Maillard reaction product) could be explained by understanding the fate of Maillard reaction products under different conditions. At atmospheric pressure (0.1 MPa), the Maillard reaction is known to occur in 3–4 stages. In the first stage, carbonyl group from a reducing sugar such as ribose reacts with amino group from the amino acid to form an imine which rearranges to the amino form (Amadori rearrangement) and subsequently is converted to volatiles and polymerized compounds (melanoidins) (Ames, 1992). The rate of Maillard reaction under these conditions strongly depends on pH, buffer, temperature, reactant concentrations, and the ratio of reactants. A buffer may exert specific effects on the reaction by acting as a catalyst (Rizzi, 2004). Studying the influence of pH on the Maillard reaction, Labuza and Baisier (1992) observed that the substrate loss increased with increasing pH, up to a pH of about 10, with little, if any, browning occurring below pH 6. Nicoli, Anese, and Lerici (1993) reported a smooth increase in Maillard browning and reaction rate constants for pH values between 2 and 8, but a rather abrupt increase between pH 8 and 10. The latter phenomenon is also obvious from the study by Ajandouz, Tchiapke, Dalle Ore, and Puigserver (2001). Furthermore, the main degradation pathways of the Amadori compound, namely enolization and retro-aldolisation, were reported to be strongly dependent on the reaction pH. Thus, M-2 marker formation during a Maillard reaction is favored by weak acidic (pH > 5.0) or alkaline (pH > 7.0) conditions. This promotes 2, 3 enolization leading to the formation of furanones (M-2) (Feather, 1981; Kim, Taub, Choi, & Prakash, 1996).

Table 3
Influence of various independent variables (pressure, temperature, time, and pH) and their interactions on chemical marker M-2 formation in whey protein gels containing 1% ribose.

Variable	Probability (P) (Effect of variables on M-2 formation)
Pressure (MPa)[a]	0.001
Temperature (°C)[a]	0.000
Time (min)[a]	0.040
pH[a]	0.000
pH × Time[a]	0.030
pH × Pressure[a]	0.028
pH × Temperature[a]	0.005
Time × Temperature[a]	0.021
Pressure × Temperature[a]	0.005
pH × Pressure × Temperature[a]	0.026
pH × Time × Temperature[a]	0.029

[a] Significant influence on decrease in the concentration of M-2 marker ($P < 0.05$).

It is well known that Maillard browning shows a strong temperature dependency. However, each intermediate step in Maillard reaction might have different temperature sensitivity. Consequently, the predominant reaction pathway involved in Maillard browning may be strongly influenced by temperature. Recently, the influence of temperature on the glucose/glycine Maillard reaction was studied, and a comprehensive kinetic model was proposed (Martins & Van Boekel, 2003). Most of the current literature on Maillard browning is primarily restricted to atmospheric pressure and very limited studies have reported the combined pressure-thermal effects on Maillard browning. This study attempts to contribute in this direction.

A comparison of M-2 yields in whey protein gels of pH = 6.1 held for any given time at 105 °C under 0.1, 350, and 700 MPa at pH of 6.1 (Fig. 2) clearly suggests that high pressure, suppressed certainsteps in a Maillard reaction. Although attempts have been made to elucidate the mechanism of inhibition and/or degradation of Maillard products under pressure (Bristow & Isaacs, 1999; Isaacs & Coulson, 1996; Tamaoka, Itoh, & Hayashi, 1991), the mechanism still stands predictive. Pressure has been proposed to suppress the condensation and browning reactions (Tamaoka et al., 1991). Use of hyperfine ESR spectra has shown that diffusion rate of unstable free radicals is greatly reduced in a Maillard system exposed to high pressures. Since several advanced steps in Maillard reaction involve the generation and participation of free radicals (Tamaoka et al., 1991), this suppression effect could have an important bearing on the fate of Maillard products. By determining the activation volume of the formation and inhibition of Maillard reaction products in model systems, it has been suggested that pressure accelerates the formation of Amadori rearrangement products and inhibits their degradation pathway (Isaacs & Coulson, 1996). Since degradation of Amadori compounds is responsible for formation of heterocyclic Maillard products and melanoidins, inhibition of the degradation of Amadori compounds would decrease the formation of Maillard reaction products. This might result in either positive or negative changes in the product sensory characteristics and consumer well being. Further studies suggest that reduction in the peak value of M-2 formed under pressure is a result of both, retardation in its rate of formation and increase in its rate of decomposition (Bristow & Isaacs, 1999).

Although pH > 5.0 and alkaline pH (pH > 7.0) favors the formation of M-2 marker, significant differences in the formation of M-2 exist as the pH is increased from 6.1 to 8.3. At higher alkaline pH values nitrogen heterocyclic products have been shown to form (Bristow & Isaacs, 1999). Likewise, as observed in the present study, the decrease in the formation of M-2 marker at pH 8.3 could be attributed to a dominating pH effect, possibly due to formation of other by-products of Maillard reaction as suggested by Bristow and Isaacs (1999).

Within the range of experimental conditions of this study, pressure, heat, pH, and time influenced the formation of M-2 marker, such that it was difficult to quantify individual effects of each of these variables on M-2. Thus, the M-2 marker was found to

be unsuitable compound for evaluating heat induced process non-uniformities during PATP. However, from food quality point of view, inhibition of certain stages of Maillard reaction under PATP could be attractive for processing certain foods. This could be especially attractive for foods in which undesirable discoloration and/or strong flavor compound formation is effected by Maillard reaction. Although M-2 marker might not be effective in mapping PATP non-uniformities, it is worth noting that other enzyme/protein based markers have been proposed for estimating thermal non-uniformities under moderate pressure–heat conditions (Gogou et al., 2010; Grauwet et al., 2010). Gogou et al. (2010) employed *Thermomyces lanuginosus* xylanase enzyme up to 600 MPa and 50–70 °C and reported synergistic effect of pressure with temperature on the enzyme inactivation. A first order inactivation kinetics was observed. Similarly, Grauwet et al. (2010) evaluated the potential of using *Bacillus amyloliquefaciens* α-amylase based indicator for mild pasteurization conditions. After exposing the above enzyme to isobaric-isothermal treatments up to 680 MPa and 10–45 °C and dynamic pressure-temperature treatments (350–600 MPa and initial temperature 10–25 °C), the enzyme activity was measured. More research is needed to develop reliable, industrially relevant sensors that can be used for mapping pressure–heat related non-uniformities especially at pressure assisted thermal sterilization conditions.

4. Conclusions

The formation of M-2 marker at atmospheric pressure was a function of temperature and holding time and was found to follow first order kinetics. However, pressure exerts a dominating effect in inhibition and/or degradation of the chemical marker M-2, thus introducing a complex effect on the formation of M-2. Also, pH has a significant influence ($p < 0.05$) on the formation of M-2 and increase in pH from 6.1 to 8.3 strongly inhibits the formation of M-2. Due to the confounding interaction of pressure with temperature in yielding M-2 marker, 4-hydroxy, 5-methyl, 3(2H) furanone (M-2) may not be a suitable marker for evaluating pressure–heat uniformity during PATP.

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