Short communication: Radio frequency dielectric heating of nonfat dry milk affects solubility and whey protein nitrogen index

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ABSTRACT

The US infant formula market is estimated at over \$3.5 billion, of which 75% are dairy-based formulas. Dried dairy powders pose a significant food safety risk, with *Cronobacter sakazakii* and *Salmonella* spp. being pathogens of particular concern. Radio frequency dielectric heating (RFDH) can provide rapid, uniform heat treatment of dry powders; thus, it potentially may be used as a postprocess lethality treatment for nonfat dry milk (NDM) or powdered infant formula. Because RFDH is a heat treatment, the functionality of the NDM may be altered and should be evaluated. High heat- and low heat-NDM were RFDH processed at temperatures ranging from 75 to 90°C for 5 to 125 min. Products were then assessed for whey protein nitrogen index (WPNI), solubility, and color. In low heat-NDM, RFDH decreased WPNI and solubility if the process was done at $\geq 80^\circ\text{C}$; however, in high heat-NDM, RFDH had a greater effect on solubility than WPNI and some color properties were altered. Further investigation of RFDH is merited to validate its application as a pathogen control process for NDM across processing parameters that result in acceptable functional properties for infant formula and other food products containing NDM.

Key words: radio frequency processing, dielectric heating, nonfat dry milk, whey protein nitrogen index

Short Communication

As an ingredient, dry milk powders provide desirable functionality (foaming and water-holding capacity) and superior nutritional properties (proteins with high biological value and minerals—such as calcium and phosphorus). The primary dry milk product made in the United States, NDM can be classified according to the extent of heat treatment before spray drying (ADPI, 2002): low heat (71°C for 2 min), medium heat (71 to 79°C for 20 min), and high heat (88°C for 30 min).

These different heat treatments affect protein denaturation, soluble whey protein nitrogen, and functional and sensory properties (Karagül-Yüceer et al., 2001; ADPI, 2002), as well as application. Nonfat dry milk is marketed for direct consumption and as an ingredient for processed foods, from ice cream and yogurt to nutritional supplements and infant formulas.

Radio frequency dielectric heating (RFDH) provides rapid and uniform heating throughout a product's mass due to friction generated by spinning polar dielectric molecules and moving ions (Zhao et al., 2000). The principles of RFDH are similar to those of microwave heating, but the frequency bands differ (13.56, 27.12, and 40.68 MHz for radio frequency vs. ~ 900 and 2,450 MHz for microwave; Rowley, 2001). Use of RFDH is more energy-efficient than other heat treatments generally used in the food industry because of the increased heat transfer efficiency, especially for low-moisture foods. In previous studies, RFDH has been used as a food safety intervention strategy (Rowley, 2001), and dry heating (storage in a hot room under controlled temperature and humidity) was shown to improve the gelling properties of dried egg white proteins (Kato et al., 1989, 1990; Mine, 1997). Heating NDM powders after spray drying and the effect on functional properties has not been explored, however. Partial denaturation of proteins in the dry form may result in improved functionality with minimal losses in solubility (Mine, 1997; Ahmed et al., 2007). The objective of this research was to determine the effect of RFDH time-temperature combinations on NDM functional properties—whey protein nitrogen index (WPNI), nitrogen solubility index (NSI), and color—by using heating parameters that might effectively reduce risks related to NDM-associated pathogens.

Spray-dried, extra grade high-heat NDM (HH-NDM) and spray-dried, extra grade low-heat NDM (LH-NDM) were obtained from ConAgra Foods (Menomonie, WI) and Dairy America (Fresno, CA), respectively. Reference standards for HH-NDM and LH-NDM were obtained from the American Dairy Products Institute (ADPI, Chicago, IL). All other reagents were laboratory-grade chemicals obtained from Fisher Scientific (Pittsburgh, PA).

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Table 1. Process time-temperature combinations¹ used for high- and low-heat nonfat dry milk

Temperature (°C)		Time (min)		
75	0	25.13	50.27	125.67
80	0	11.55	23.10	57.75
85	0	5.00	10.00	25.00
90	0	2.30	4.60	11.50

¹Based on preliminary work conducted to achieve 1, 2, and 5 log reductions in *Salmonella* spp. and *Cronobacter sakazakii* in nonfat dry milk.

Samples of NDM were analyzed for ash (AOAC International, 2002; method 942.05), moisture (ADPI, 2009b), protein (IDF, 2002b) and fat (AOAC International, 2002; method 942.39) contents, WPNI (ADPI, 2009b), and NSI (IDF, 2002a). The NSI method was modified to deliver 3.5 g of protein/100 mL. Nitrogen was analyzed with a Leco FP-2000 protein analyzer (Leco Corp., St. Joseph, MI), and protein was calculated using a multiplication factor of 6.38 (IDF, 2006).

Surface color of milk powders was measured with a Hunter Lab MiniScan EZ 4500L spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) with data reported on the CIE L*, a*, and b* scale [L* represents lightness from black (0) to white (100), a* represents red (+) or green (−), and b* indicates yellow (+) or blue (−)]. To measure color, milk powder was placed in a plastic petri dish (100 × 15 mm; BD Falcon Fisher Scientific, Denver, CO) to a depth of approximately 3 mm, and triplicate measurements were done on each sample after defined time-temperature heat treatments.

Because no previous research has been reported on RFDH of NDM, a preliminary study was done to design the RFDH treatment (data not presented). Process time-temperature combinations (Table 1) were selected based on this preliminary work and focused on achieving 1, 2, and 5 log-cycle reductions in *Salmonella* spp. and *Cronobacter sakazakii* populations in NDM (data not presented). About 330 g of HH-NDM or 450 g of LH-NDM was placed in a high-density polyethylene container (0.3 cm thick, 22.4 cm in diameter, and 2.1 cm high; Dynalab Corp., Rochester, NY). A fiber optic probe (T1, Neoptix Inc., Québec, QC, Canada) was used to monitor the sample core temperature, and temperature was recorded using a data logger (Reflex, Neoptix Inc.). Samples were heated in the 6-kW RFDH unit (Strayfield Ltd., Theale, UK) operating at a frequency of 27.12 MHz. Due to the dry nature of the NDM (~3% moisture), maximum radio frequency was not coupled with more than 3 kW power and operating electrode gap of 120 mm. When the target core temperature was reached, RFDH power was turned off and the heated sample was transferred to a convection oven (Thelco, GCA/Precision Scientific, Schaar

Scientific Co., Chicago, IL) preset at the designated temperature and held for the appropriate time (Table 1). Samples were removed, placed in 16.5 × 14.9 cm resealable plastic bags (Ziploc, S.C. Johnson & Son, Racine, WI), cooled to 25°C ± 2°C, and then sealed. Samples were stored at −2°C until analysis.

The experiment was conducted as a randomized block with 3 replications. Each NDM type (HH or LH) was tested in a separate experiment. The NDM samples that were not RFDH-treated served as control treatments. Data were analyzed with SAS version 9.2 (SAS Institute Inc., Cary, NC) using a one-way ANOVA, with significance determined at $P \leq 0.05$ for treatments. Significant means were differentiated with the Dunnett test, which compares treatments to a control while minimizing comparison-wise error rates (Kuehl, 2000).

Table 2 displays the compositional values, NSI, and WPNI results of HH- and LH-NDM. Although ash content was slightly lower than expected, the composition and classification of these samples complied with standards (ADPI, 2002). Nonfat dry milk is classified into low heat, medium heat, and high heat to reflect the degree of the milk's heat treatment before spray drying, according to the WPNI assay (ADPI, 2002, 2009a). The WPNI values in Table 2 for LH-NDM (>6 mg/g) and HH-NDM (<1.5 mg/g) verify the stated classification of the source materials (ADPI, 2002).

The time required for NDM to increase from ~22°C to the target RFDH temperatures (75, 80, 85, or 90°C) ranged from 4.18 to 6.60 min. Results of the ANOVA indicated that RFDH affected NSI in both HH-NDM and LH-NDM ($P \leq 0.05$). Mean comparisons are shown in Table 3. For HH-NDM, all RFDH-treated samples had significantly lower solubility than the control, except for HH-NDM treated at 75°C at 0 min. Significant NSI losses ranged from 1.3 to 3%. In contrast, RFDH caused a significant loss (ranging from 3 to 5%) in NSI of LH-NDM treated at ≥80°C. The sample of LH-NDM that was RFDH-treated at 75°C had similar NSI compared with the control, regardless of process time. As expected, the overall NSI values of LH-NDM were greater than those of HH-NDM (Tables 2 and 3), confirming that the pre-spray-drying treatment affects

Table 2. Composition of high-heat and low-heat NDM¹

Item	HH-NDM ²	LH-NDM ²
Lactose (%)	53.52 ± 0.02	54.93 ± 0.04
Protein ³ (%)	35.36 ± 0.01	34.33 ± 0.06
Ash (%)	7.69 ± 0.03	7.82 ± 0.01
Moisture (%)	3.36 ± 0.01	3.42 ± 0.02
Fat (%)	0.07 ± 0.01	0.03 ± 0.01
NSI ⁴ (%)	90.6 ± 0.5	93.3 ± 0.4
WPNI ⁵ (mg/g)	1.48 ± 0.02	7.24 ± 0.06

¹Mean ± SEM, n = 3.²HH-NDM = high-heat nonfat dry milk, LH-NDM = low-heat nonfat dry milk.³Used 6.38 as conversion factor from total nitrogen (%N × 6.38).⁴Nitrogen solubility index.⁵Whey protein nitrogen index.

solubility (Singh, 2009). The loss in solubility may be due to conformational changes in the proteins and needs to be studied further to understand the true effect on the protein (Robin et al., 1993).

Treatment with RFDH had little effect on the WPNI of HH-NDM; WPNI ranged from 1.32 (85°C for 25 min) to 1.48 mg/g (control). For HH-NDM, RFDH significantly reduced WPNI in 3 samples compared with the control: 80°C for 57 min, 85°C for 10 min, and 85°C for 25 min (Table 4). For the LH-NDM, WPNI means ranged from 7.32 (75°C for 125 min) to 5.87 mg/g (sample treated at 85°C for 25 min); RFDH significantly reduced WPNI in all LH-NDM if the temperature was ≥80°C. The significant reduction in WPNI ranged

from 11 to 19%. Although significant reductions were noted, all RFDH-treated LH-NDM samples remained in the low-heat classification (ADPI, 2009b) except the sample treated at 85°C for 25 min. However, the LH-NDM control (untreated) had a relatively large portion of nondenatured whey protein (WPNI = 7.167 mg/g). Although the increased WPNI of some treated NDM might suggest that denaturation decreased, this phenomenon is probably due to dissociation of α-LA and β-LG from the casein micelles (Dagleish et al., 1997; Dannenberg and Kessler, 1998).

Previous studies on thermal denaturation of whey proteins in aqueous systems have confirmed that, in pH 6.5 to 7.0 systems, α-LA, BSA, and β-LG begin to denature within a range from 61 to 67, 62 to 78, and 70 to 80°C, respectively (Paulsson and Dejmak, 1990). Other researchers have suggested that increased whey protein denaturation reduces WPNI and solubility (Pelegrine and Gasparetto, 2005). An interesting observation was that if LH-NDM (~3.2% moisture) was RFDH-treated at 75°C for ~2 h, WPNI and NSI were similar to those of the control, indicating that minimal moisture contents perhaps delayed the onset of whey protein denaturation.

Because RFDH is a heat treatment, and the NSI and WPNI results indicated that protein changes occurred, color was monitored to determine if other reactions had been initiated—such as Maillard browning (Moraes and van Boekl, 1998). The sample of HH-NDM that was RFDH treated at 90°C for 0 min had an in-

Table 3. Dunnett's mean¹ comparison results for solubility of high-heat (HH) and low-heat (LH) nonfat dry milk (NDM) samples

Temperature (°C)	Time (min)	NSI ² (%)	
		HH-NDM	LH-NDM
Control ³	—	90.6 ± 0.5	93.3 ± 0.4
75	0	90.1 ± 0.3	93.0 ± 0.6
75	25.3	89.4 ± 0.2 ^a	93.2 ± 0.2
75	50.27	89.5 ± 0.2 ^a	92.6 ± 0.3
75	125.67	89.2 ± 0.1 ^a	93.1 ± 0.2
80	0	89.0 ± 0.2 ^a	89.4 ± 0.5 ^a
80	11.55	88.9 ± 0.1 ^a	89.1 ± 0.4 ^a
80	23.10	88.9 ± 0.2 ^a	88.8 ± 0.2 ^a
80	57.75	88.3 ± 0.3 ^a	89.2 ± 0.5 ^a
85	0	88.9 ± 0.1 ^a	90.1 ± 0.9 ^a
85	5.00	88.6 ± 0.4 ^a	88.6 ± 0.3 ^a
85	10.00	88.5 ± 0.1 ^a	88.4 ± 0.1 ^a
85	25.00	88.2 ± 0.1 ^a	88.4 ± 0.2 ^a
90	0	88.1 ± 0.5 ^a	89.1 ± 0.4 ^a
90	2.30	88.3 ± 0.2 ^a	88.8 ± 0.2 ^a
90	4.60	88.0 ± 0.1 ^a	88.5 ± 0.1 ^a
90	11.50	87.9 ± 0.3 ^a	88.3 ± 0.3 ^a

^aMeans with a superscript within a column differ from the control ($P \leq 0.05$).¹Mean ± SEM, n = 3.²Nitrogen solubility index.³Nontreated sample.

Table 4. Dunnett's mean¹ comparison results for whey protein nitrogen indices (WPNI) of high-heat (HH) and low-heat (LH) nonfat dry milk (NDM) samples

Temperature (°C)	Time (min)	WPNI (%)	
		HH-NDM	LH-NDM
Control ²	—	1.48 ± 0.02	7.24 ± 0.06
75	0	1.44 ± 0.02	7.18 ± 0.04
75	25.13	1.45 ± 0.03	7.24 ± 0.06
75	50.27	1.45 ± 0.02	7.16 ± 0.11
75	125.67	1.48 ± 0.02	7.32 ± 0.05
80	0	1.39 ± 0.03	6.46 ± 0.09 ^a
80	11.55	1.38 ± 0.04	6.48 ± 0.11 ^a
80	23.10	1.33 ± 0.01	6.44 ± 0.02 ^a
80	57.75	1.34 ± 0.01 ^a	6.35 ± 0.04 ^a
85	0	1.45 ± 0.01	6.22 ± 0.06 ^a
85	5.00	1.45 ± 0.02	6.11 ± 0.05 ^a
85	10.00	1.35 ± 0.06 ^a	6.05 ± 0.13 ^a
85	25.00	1.32 ± 0.03 ^a	5.87 ± 0.12 ^a
90	0	1.44 ± 0.04	6.31 ± 0.13 ^a
90	2.30	1.44 ± 0.04	6.05 ± 0.04 ^a
90	4.60	1.38 ± 0.04	6.05 ± 0.08 ^a
90	11.50	1.38 ± 0.03	6.06 ± 0.02 ^a

^aMeans with a superscript within a column differ from the control ($P \leq 0.05$).

¹Mean ± SEM, n=3.

²Nontreated sample.

creased b* value, indicating a more yellow color (Table 5). However, in the LH-NDM experiment, 3 RFDH-treated samples had a significant color change (Table 6): samples treated at 75°C for 25 min and 85°C for 10 min were greener, whereas that treated at 90°C for 0 min resulted in a yellower product than the control. The b* value reflects color changes toward yellow and

brown (Morales and van Boekl, 1998; Outinen et al., 2010), suggesting that browning (Maillard reaction) might have been initiated during the RFDH treatment.

Treatment of NDM by RFDH reduced WPNI and protein solubility, and these effects were functions of the process temperature and time. After comparing the effects of RFDH at 75, 80, 85, and 90°C, the 90°C

Table 5. Dunnett's mean¹ comparison results for color attributes (L*, a*, and b*) of high-heat nonfat dry milk (NDM) samples

Temperature (°C)	Time (min)	Color ²		
		L*	a*	b*
Control ³	—	83.35 ± 5.43	−2.53 ± 0.15	11.46 ± 0.78
75	0	84.76 ± 3.26	−2.45 ± 0.30	11.97 ± 0.80
75	25.13	85.83 ± 0.46	−2.68 ± 0.22	11.77 ± 0.49
75	50.27	83.00 ± 0.68	−2.40 ± 0.17	12.11 ± 0.18
75	125.67	85.72 ± 1.61	−2.58 ± 0.21	11.91 ± 0.42
80	0	83.37 ± 2.49	−2.71 ± 0.20	11.80 ± 0.72
80	11.55	84.26 ± 0.83	−2.41 ± 0.27	12.17 ± 0.26
80	23.10	85.28 ± 0.85	−2.66 ± 0.24	12.27 ± 0.17
80	57.75	84.36 ± 1.83	−2.46 ± 0.17	12.24 ± 0.22
85	0	87.77 ± 1.71	−2.75 ± 0.12	12.65 ± 0.86
85	5.00	84.98 ± 0.79	−2.65 ± 0.02	12.36 ± 0.16
85	10.00	86.50 ± 2.28	−2.52 ± 0.13	12.57 ± 0.36
85	25.00	86.74 ± 0.45	−2.57 ± 0.18	12.18 ± 0.16
90	0	85.44 ± 2.26	−2.61 ± 0.24	13.27 ± 0.80 ^a
90	2.30	86.99 ± 1.00	−2.67 ± 0.40	12.40 ± 0.24
90	4.60	84.47 ± 4.55	−2.67 ± 0.12	12.81 ± 0.40
90	11.50	84.83 ± 1.15	−2.54 ± 0.21	12.95 ± 0.78

^aMeans with a superscript within a column differ from the control ($P \leq 0.05$).

¹Mean ± SEM, n = 3.

²*represents lightness from black (0) to white; a* represents red (+) or green (−); b* represents yellow (+) or blue (−).

³Nontreated sample.

Table 6. Dunnett's mean¹ comparison results for color attributes (L*, a*, and b*) of low-heat nonfat dry milk (NDM) samples

Temperature (°C)	Time (min)	Color ²		
		L*	a*	b*
Control ³	—	78.56 ± 6.95	-1.89 ± 0.25	12.27 ± 1.21
75	0	82.78 ± 1.31	-2.28 ± 0.27	13.05 ± 0.38
75	25.13	78.18 ± 1.52	-2.51 ± 0.10 ^a	12.92 ± 0.87
75	50.27	80.65 ± 2.40	-2.05 ± 0.12	12.59 ± 0.50
75	125.67	79.86 ± 0.56	-2.19 ± 0.22	13.60 ± 0.93
80	0	79.817 ± 1.41	-1.83 ± 0.22	12.84 ± 1.39
80	11.55	78.01 ± 3.17	-1.95 ± 0.30	13.10 ± 0.95
80	23.10	80.68 ± 2.75	-2.34 ± 0.31	13.71 ± 0.30
80	57.75	80.78 ± 3.10	-2.24 ± 0.08	12.97 ± 0.57
85	0	78.25 ± 3.14	-1.97 ± 0.06	12.80 ± 0.94
85	5.00	82.48 ± 5.12	-2.17 ± 0.10	13.34 ± 0.34
85	10.00	81.90 ± 1.16	-2.44 ± 0.26 ^a	13.66 ± 0.88
85	25.00	79.08 ± 2.18	-1.93 ± 0.33	12.67 ± 0.92
90	0	78.89 ± 6.14	-2.06 ± 0.29	14.43 ± 0.83 ^a
90	2.30	79.45 ± 2.06	-2.31 ± 0.21	13.14 ± 0.57
90	4.60	79.67 ± 0.14	-2.16 ± 0.10	14.00 ± 0.95
90	11.50	81.64 ± 1.74	-2.11 ± 0.17	12.27 ± 1.21

^aMeans with a superscript within a column differ from the control ($P \leq 0.05$).

¹Mean ± SEM, $n = 3$.

²*represents lightness from black (0) to white; a* represents red (+) or green (-); b* represents yellow (+) or blue (-).

³Nontreated sample.

treatment was deemed more practical than the others. Although the longest holding time was ~12 min, this treatment induced an approximately 16% reduction of WPNI in LH-NDM, but the final value was greater than 6 mg/g, maintaining the LH-NDM classification. This RFDH treatment also induced about 6% solubility loss, which could affect other NDM functional properties, such as foaming or emulsifying. This study focused on using a nontraditional technology in the dairy foods industry that may have applications for microbial control. However, when assessing new technologies that may provide microbial control, it is crucial to quantitatively characterize potential positive and negative effects on the functional properties and component interactions in the product, in this case NDM.

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