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Improving the Formation, Stabilization, Quality, and Extending the Shelf Life of Camel Meat Emulsion using Chitosan

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Abstract

The main objective of the current study was to apply chitosan as a stabilizing material to improve the quality and extend the shelf life of camel meat emulsion. Chitosan may achieve these roles through the adsorption of the protective layer at oil-water interfaces and viscosity enhancement. The addition of chitosan at a concentration of 0.8% resulted in overcoming the detrimental impact of the heat-stable camel connective tissue and improve emulsion stability. The obtained results revealed that the addition of chitosan significantly decreased the total expressible fluid, released water, released fat and Thiobarbituric Acid Reactive Substances (TBARS) of raw camel meat batter with a significant increase in the batter's viscosity and water Holding Capacity (WHC) compared to the control. After cooking, the addition of chitosan led to significant reduction of TBARS value and extended the shelf life of camel sausage to 5 months at 5°C when compared to the control that accepted only for 3 months. Moreover, the chitosan formulated sausage showed lower lightness (L*), yellowness (b*) and shear force values than the control throughout chilled storage at 5°C for 5 months. Improvement of redness (a*), the sensory parameters and ultrastructure of camel luncheon sausage was also observed by the chitosan addition. Therefore, chitosan can be applied as a thickener and stabilizer for camel meat emulsion and produce more stable products through 5 months of refrigerated storage. Furthermore, the addition of chitosan may be safely used by meat processors as a novel technique for improving the quality and extending the shelf life of camel luncheon.

KEYWORDS Camel luncheon, Chitosan, Emulsion, Stabilization.

INTRODUCTION

Camel comes first for the production of meat in arid and semiarid areas where other animals cannot withstand adverse climatic conditions (Kadim *et al.*, 2006). In general, camel meat is very popular and is in vital demand in many Asian and African countries, however, many consumers found it less juiciness, very tough, and less palatable (Soltanizadeh *et al.*, 2008). The lower sensory quality of camel meat mostly decreases its acceptability as a table meat, which makes its processing into a variety of products an acceptable trend (Kadim *et al.*, 2008). Emulsion-type products constitute a major sector in the meat industry; therefore, the processing of highly specialized emulsion-type products from organoleptically unacceptable camel meat can be made affordable to low-income societies in underdeveloped and developing countries (Kadim *et al.*, 2013).

The production of a successful meat emulsion is a crucial issue (Santhi *et al.*, 2015) because it is generally thermodynamically unstable and will finally break down. Therefore, a correct emulsion stabilizer must be added to improve the stability and shelf life of the finished product (McClements, 2005). The polysaccharides are used to increase the emulsion stability of meat batters containing proteins (Dickinson, 2011; Murray, 2011; Schmitt and Turgeon, 2011) and are often more effective in improving emulsion stability than either surfactants or proteins (McClements, 2005).

Chitosan is the second most abundant polysaccharide in nature. The incomparable properties e.g., nontoxic, non-allergenic (Kumar et al., 2004), good biodegradability, and biocompatibility make chitosan a promising biopolymer for commercial applications in a broad range of food products (Tharanathan and Kittur, 2003). The ability to transform into gels and colloids (Coma et al., 2002; Senel and McClure, 2004), as well as the non-digestibility and the bland taste, are additional exclusive characteristics of chitosan that make it an excellent food additive (Chhabra, 2004). The most important food applications of chitosan are edible film coatings (Elsabee and Abdou, 2013), color stabilization (Suman et al., 2010), antimicrobial (Benhabiles et al., 2012), thickening, gelling, and emulsifying agent (Li and Xia, 2010; Klinkesorn, 2013). Although, the use of chitosan as an emulsion stabilizer becomes a novel trend particularly in flavor oil (Kaasgaard and Keller, 2010), there are no available data on its application in meat emulsions. Moreover, the available data about the technological properties and quality attributes of emulsion types products that processed by camel meat are very scarce. Therefore, the principal goal of the present study was to evaluate the suitability of chitosan as an emulsion stabilizer to overcome the difficulties facing the successful production of camel meat emulsion due to its high con-

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tent of heat-stable connective tissue.

MATERIALS AND METHODS

Raw materials

The silver side muscles and the hump fat of 5-years old Arabian male camels (*Camelus dromedarius*) were obtained from El-Bassaten slaughterhouse (Cairo, Egypt) after elapse of 4 hours post-slaughter. Both meat and fat were kept at 4 °C for 24 hours. Sodium nitrite was purchased from BASF, Chemical Company (Ludwigshafen Rhine, Germany), Sodium tripolyphosphate was obtained from FOODCHEM Company (Shanghai, China), Spice oleoresins were obtained from Nubassa GewürzwerkGmpH (Viernheim, Germany), and Chitosan-low molecular weight-Synonym: Deacetylated chitin, Poly (D-glucosamine) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Common salt and corn starch were purchased from the local market at Giza, Egypt.

Processing of camel emulsion-type sausages

The control meat batter was produced in triplicate without the addition of chitosan following Good Manufacturing Practices. For the production of 100 kg, 65kg camel meat, 16 kg hump fat, 12 kg iced water, 5 kg cornstarch, 1.6 kg common salt, 10 g sodium nitrite, 300 g sodium tripolyphosphate, and quantum sufficient of the spice mixture (Oleoresins of coriander, mace, garlic, cardamom, white pepper, and capsicum) was used. Both lean and fat were minced immediately before the preparation of meat batter using a Laska meat grinder (K 65; Gesellschaft m.b.H Maksrtstraße 60, A-4050 Traun/Austria) at 5 mm mincing plate. The Lean was then chopped in K-65 Laska bowl cutter for 2 min with common salt, sodium tripolyphosphate, sodium nitrite, and spice mixture at 4000 × g before iced water was added. The ground fat was added just before -1°C and chopped till achieve batter's temperature of 2°C, and finally, the starch was added and chopped to 7 °C final temperature. The chitosan-incorporated batter was also replicated three times with the addition of 0.8% chitosan. The prepared batters were stuffed separately into 90mm Viskase polyamide casing (Walsroder, GmbH, Germany), left for a couple of hours at 4°C, then cooked using Maurer-Atmos oven (Middleby GmbH Kindlebindstr 100-D78479, Germany). A complete humid cooking program (started with 65°C for one hour followed by gradual increase in temperature to 75°C for another one hour then finally, to 85°C to achieve 75°C core temperature) was used. After cooking, showering was performed for one hour with half an hour of rest in-between.

Investigations

Three samples from each replicate were analyzed for emulsion stability, batter viscosity, and WHC before thermal treatment and for instrumental color indexes, shear force, and sensory investigation after cooking and during storage at 5 °C for 5 months. Determination of TBARS and Scanning Electron Microscopy were performed for both raw batters and cooked sausages. All investigations were run three times for each sample.

Emulsion stability

The emulsion stability parameters of different camel meat batters (raw sausages) were evaluated using the method of Colmenero *et al.* (2005). Twenty-five grams from each prepared meat batter were centrifuged at 6000 rpm for 15 min at 4°C using a cooling centrifuge (Beckman Coulter, Indianapolis, United States). After centrifugation, the sample was heated at 70°C in a water bath for 30 minutes and then centrifuged at 6000 rpm for 20 min. The percentage of the total fluid released (TFR) was calculated in relation to the initial weight, and the loss in the TFR after heating at 105°C in a hot air oven for 15 hours is reported as the water released (WR), while the difference between TFR and WR is the percentage of fat released (FR).

Batter viscosity

The viscosity of the different meat batters was measured three times for each sample following the procedure of Jafarpour and Gorczyca (2009) using a Rheostress RS50 rheometer (HAAKE, Germany) with a plate (MP60 steel 18/8) and cone (60 mm $\emptyset/2$ angle). Five grams of each sample was loaded to the rheometer between the cone and the plate and left for five min at 10°C. The sample was heated (10-80°C) with 1°C/min heating rate, 100 pa stress, and 0.1 Hz frequency values.

Water-Holding Capacity (WHC)

The centrifugation method as described by Zheng *et al.* (2017) was used to determine the Water Holding Capacity of camel meat batters. Ten grams of each sample was centrifuged at 4°C for 20 min at 20.000xg in Beckman Coulter cooling centrifuge. The WHC% was calculated from the weight of the supernatant to the initial sample weight.

Instrumental color measurement

Changes in the instrumental color indexes of cooked sausage were evaluated using a Konica Chroma meter (CR 410, Japan) equipped with D-625 illuminant with a 52 mm diameter sphere size, 8 mm /11 mm aperture size and 10° observer angle and calibrated with a white plate (L* = +97.83, a* = 0.43, b* = +1.98) (Shin *et al.*, 2008). After 30 min bloom time, the color indexes of each sausage sample were measured from the longitudinal cut surfaces of the sausages using a portable colorimeter. Three slices from each loaf were used and six reading for each of the lightness (L*), redness (a*), and yellowness (b*) were obtained from the surface of each slice at each time of analysis and the average score of replicates was recorded and expressed as CIE (L*), (a*), and (b*).

Measurement of Thiobarbituric Acid Reactive Substances (TBARS)

The lipid oxidation of raw camel batters and cooked sausages was evaluated by determining TBARS in triplicate for each sample following the method of Du and Ahn (2002). Five grams of batter and luncheon sausage from each trial was thoroughly homogenized with 15 mL double distilled water for 10 seconds at the highest speed. One ml sample homogenate was mixed with 50 ul of 7.2% butylated hydroxytoluene and 2 ml TBA-trichloroacetic acid (15 mM TBA-15% TCA). The mixture was heated in boiling water for 15 min, cooled for 10 min in an ice bath then centrifuged at 2500xg for 15 min. The absorbance of the supernatant was measured at 531 nm using a blank (1 mL deionized water and 2 mL of TBA-TCA), and the mean value of TBARS was expressed as milligrams malonaldehyde/kg.

Shear force

Shear forces of control and chitosan-treated camel luncheon

sausage were determined by the method of Shackelford *et al.* (2004). Two cubes (2 x 1 x 1 cm) were sampled from each sausage loaf and sheared three times using a Warner–Bratzler shear force device and an Instron Universal Testing Machine (Instron Corp., Canton, MA, USA) with a 55-kg tension/compression load cell and a cross-head speed of 200mmmin–1.

Scanning electron microscope

Three 1 x 1 x 2 cm pieces from each sample were fixed in 2.5% phosphate-buffered glutaraldehyde at 4°C for 2 h, rinsed three times (30 min each) in phosphate buffer saline (0.1M), and dehydrated in an upgrading series ethanol (Ketnawa and Rawdkuen, 2011). Samples were then dried using CO_2 as a transition fluid for 15 min. in (Sandri-PV-3D) critical point drier for 15 minutes, followed by gold coating in a vacuum evaporator (Jeol JFC 1100 E) and examined under QUANTA FEG 250 SEM Hillsboro Scanning Electron Microscopy (Oregon, USA).

Sensory investigations

Scores for sensory samples for both control and chitosan-integrated luncheon sausage were obtained from 90 untrained panelists across 2 sessions and 3 nights (6 sessions in total). Each sample was sub-sectioned into 5 samples and served to the panelists following the guidelines of the American Meat Science Association (AMSA, 2015). Briefly, 180 samples were sliced at 2 mm thickness, where 15 participants in each session evaluated randomly tested 5 samples. Before the panel sessions, participants were asked for personal information regarding, income, sex (male or female), frequency of luncheon consumption per month, and their preferred characteristics. The personal information was later converted into ordinal categories for use in statistical analysis. All testing was carried out under controlled conditions with tap water available to cleanse the mouth between samples. Each panelist was asked to evaluate each luncheon sample for appearance, binding, flavor, juiciness, tenderness, and overall acceptability using 1-9 hedonic scale, where 9 indicates extremely acceptable and 1 indicates extremely unacceptable.

Statistical analysis

The camel sausage production was replicated three times on three different days and measurements of related investigations were conducted in triplicate for each batch. For each formulation, three raw (meat batter) and cooked sausage samples per batch were randomly selected for analysis. Variation in physicochemical criteria of meat batters (emulsion stability, batter viscosity, WHC, and TBARS) was described using linear mixed models (LMM), with processing treatments (control and chitosan) as a fixed effect and random effect for batches. Moreover, TBARS, instrumental color indexes, and shear force of cooked camel sausages during storage for 5 months were analyzed in a similar fashion. In each LMM, the processing treatments and sampling months (0, 1, 3, and 5) were considered as a fixed effect while, batches were considered as a random effect. Approximate F-ratio tests for each fixed effect were conducted and critical values for a statistically important effect taken at P<0.05. Predicted means and standard errors were generated from the models. Pairwise comparison between means was enabled by calculation of least significant difference at 5% critical value. For the sensory analysis (appearance, binding, flavor, juiciness, tenderness, and overall acceptability), fixed terms included processing treatment and time of examination. The experimental design factors of batches, panelists, and sessions were fitted as random effects. Predicted means, standard errors, and least significant difference at 5% critical value of each trait were predicted from each processing treatment. All statistical modeling and presentations were conducted using the SPSS software for windows (SPSS 23.0 for Windows; SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

It is crucial to improve the meat emulsion characteristics e.g. emulsion stability, WHC, color, texture, and fat stability to increase the acceptance of the finished product. The obtained data clarified that chitosan can successfully overcome the detrimental impact of the heat-stable camel connective tissue and improve emulsion stability. The addition of chitosan significantly decreased (P < 0.05) the total expressible fluid, released water, and released fat of camel meat batter. It also increased the batter viscosity from 208092.9 to 298570.1 Pas and the WHC from 94.7 to 98.6% (Table 1). The obtained results were in agreement with Pranoto et al. (2005) and Cutter (2006). Chitosan can act as an emulsion stabilizer in the meat system by increasing the viscosity of the continuous aqueous phase which slows down the diffusion of fat droplets (Calero et al., 2010; Klinkesorn, 2013). Moreover, chitosan can improve the gelling property (Agulló et al., 2003), the formation of thick and strong multilayers around the oil droplets (McClements, 2005; Calero et al., 2010), and the reduction of the oil-water interfacial tension ultimately prevents the flocculation, aggregation, and coalescence of the fat droplets (Kandeepan et al., 2013) which support the finding of the current study.

Chitosan showed a pronounced antioxidant effect and delayed the lipid oxidation of camel meat emulsion immediately after processing and during chilled storage. The TBARS values

Table 1. Predicted means of emulsion stability% (total fluid released, TFR; fat released, FR and water release, WR), batter viscosity (Pa·s), water holding capacity, WHC (%), and thiobarbituric acid reactive substances, TBARS (milligrams malonaldehyde/kg) for meat batters from each treatments (control and chitosan)

	Control	Chitosan	Standard error
Emulsion stability			
TFR%	16.1ª	8.6 ^b	0.19
FR%	7.6ª	6.6 ^b	0.07
WR%	8.5ª	2.0 ^b	0.13
Batter viscosity (Pa·s)	208092.9ª	298570.1 ^b	3414.74
WHC%	94.7ª	98.6 ^b	0.6
TBARS (milligrams malonaldehyde/kg)	0.43ª	0.29 ^ь	0.01

Differences between means with different superscripts exceed the estimate of least significant difference at 5% critical value. Comparisons were made on an individual trait level and are not applicable down rows.

of raw batter were reduced from 0.44 to 0.36 mg malonaldehyde per kilogram by addition of chitosan (Table 1). The results also clarified that TBARS value of the control sausage exceeded the permissible limit (0.90 mg malonaldehyde per kilogram, EOS/1114, 2005) while, that of chitosan formulated sausage was lower than the permissible limit at the end of 5 months of storage at 5°C (Table 2). The obtained antioxidant activity of chitosan was in good agreement with Fan *et al.* (2009) who observed a distinct inhibition of lipid oxidation in chitosan-coated fish during frozen storage. The antioxidant activity of chitosan is probably due to the reaction of its amino groups with the volatile aldehydes derived from fat oxidation e.g., malonaldehyde with subsequent formation of stable compounds (Coma *et al.*, 2002). Moreover, the antioxidant function of chitosan is determined by its molecular weight as relatively low molecular weight chitosan showed a higher antioxidant capacity than high molecular weight chitosan

Table 2. Predicted means of thiobarbituric acid reactive substances, TBARS (milligrams malonaldehyde/kg), instrumental color (lightness, L*; redness, a* and yellowness,), and shear force (Newtons) for cooked camel luncheon sausages from each treatments (control and chitosan) during chilled storage at 5°C for 5 months

	0-time	1m	3m	5m	Standard error
TBARS (milligrams malona	aldehyde/kg)				
Control	0.44ª	0.51°	0.63 ^d	0.95°	0.01
Chitosan	0.36 ^b	0.42ª	0.49°	0.52°	-
Instrumental color					
L^*					
Control	52.3 ^{ab}	52.7 ^{ac}	52.1 ^{ab}	52.5 ^{abc}	0.33
Chitosan	53.3°	51.7 ^{bd}	51.2 ^{de}	50.8°	-
<i>a</i> *					
Control	18.5ª	19.5 ^{cd}	19.3°	19.3°	0.1
Chitosan	21.3 ^b	21.6 ^b	19.6 ^d	19.4 ^{cd}	-
b^*					
Control	10.3ª	10.6°	11.1 ^d	12.2°	0.07
Chitosan	9.9 ^b	10.4 ^{ac}	11.0 ^d	12.6 ^f	-
Shear force (Newtons)					
Control	41.5ª	51.2°	79.3°	79.1°	0.44
Chitosan	45.4 ^b	47.5 ^d	68.3 ^f	69.5 ^g	-

Differences between means with different superscripts exceed the estimate of least significant difference at 5% critical value. Comparisons were made on an individual trait level and are applicable across rows and columns within each trait (TBARS, instrumental color and shear force).

Table 3. Predicted means of sensory	scores for cooked camel	luncheon sausages fro	om each treatments	(control and chitosan) of	during chilled storage	e at 5°C for 5
months						

	0-time	1m	3m	5m	Standard error	
Appearance						
Control	7.3 ^{ab}	7.3 ^{ab}	7.2 ^{ad}	7.0^{d}	0.08	
Chitosan	8.3°	8.0^{d}	7.7°	7.5 ^{be}	-	
Binding						
Control	7.7ª	7.0°	6.7 ^d	6.3 ^f	0.09	
Chitosan	8.0 ^b	7.7ª	7.3°	7.2 ^{ce}	-	
Flavor						
Control	7.3ª	6.0°	6.0°	5.3 ^d	0.06	
Chitosan	8.0 ^b	7.3ª	7.2ª	6.7 ^e	-	
Juiciness						
Control	7.0ª	6.3°	6.3 ^{ce}	6.2 ^e	0.12	
Chitosan	7.7 ^ь	7.5 ^{bdf}	7.3 ^{af}	7.3 ^{ad}	-	
Tenderness						
Control	$7.7^{\rm abc}$	7.2°	6.7 ^{de}	6.3°	0.11	
Chitosan	8.0 ^b	7.7 ^{ab}	7.3 ^{ac}	7.2 ^{cd}	-	
Overall acceptability						
Control	7.5 ^{acd}	7.2 ^{ce}	6.9 ^{cf}	6.5 ^f	0.09	
Chitosan	8.3 ^b	7.9 ^d	7.5 ^{ed}	7.1 ^{ac}	-	

Differences between means with different superscripts exceed the estimate of least significant difference at 5% critical value. Comparisons were made on an individual trait level and are applicable across rows and columns within each trait (appearance, binding, flavor, juiciness, tenderness and overall acceptability) (Wu and Mao, 2009).

The data for the instrumental color of cooked camel luncheon sausage during storage at 5°C for 5 months (Table 2) indicated a significant effect (P < 0.05) of chitosan on L*, a*, and b* values. The lightness "L*" values of the control camel sausages significantly increased (P < 0.05) during storage in comparison with the samples prepared with chitosan. The high water binding ability of chitosan decreased the released water (Table 1) and lowered the lightness values (Fischer, 2007). The redness "a*" values of both luncheon sausage treatments significantly increased (P < 0.05) during storage for 5 months in addition, the chitosan incorporated samples showed significant higher a* values (P < 0.05) at zero time and throughout chilled storage for 5 months compared with the control samples. The results also revealed that addition of chitosan during processing of camel luncheon sausage led to significant reduction (P < 0.05) in yellowness "b*" values at zero time and throughout the chilled storage for 5 months when compared with the control. Sayas-Barberá et al. (2011) found that chitosan enhanced the red color of a "burger" model system. Moreover, Goycoolea et al. (2009); Badawy and Rabea (2017) found that the antioxidant effect of chitosan lowered the color oxidation of meat and decreased the met-myoglobin formation with the consequent increase in redness and decrease in yellowness values that preserves and stabilizes the color of the finished product. This observation suggested that chitosan generally improved the color of camel luncheon sausage. The results also clarified that addition of chitosan led to significant reduction (P < 0.05) of the shear force values at 1st month till 5th months of chilled storage in comparison with control (Table 2). Shear force results may be explained by the higher WHC and lower released water percentage in luncheon sausage processed with chitosan than control group (Table 1). The obtained data substantiated the results of sensory analysis where, tenderness and juiciness scores were significantly higher (P < 0.05) in chitosan incorporated sausage than those of control (Table 3) either after processing or throughout storage period.



Fig.1. Scanning Electron Micrographs of camel meat batter. A, Control (x500), B control (x2000); C with chitosan (x500), D with chitosan (x2000).

Sensory evaluation was carried out for camel luncheon sausages during chilled storage for 5 months at 5°C (Table 3). It has been noticed that the addition of chitosan to camel luncheon sausage caused significant improvement of all sensory parameters in comparison to the control after processing (0-time) and during storage. The antioxidant effect of chitosan and the consequent lower TBARS of the luncheon sausage prepared with chitosan (Tables 1,2), thereby caused a more acceptable flavor and overall acceptability. Moreover, the high lipid and water-binding capacities of chitosan (Table 1) largely explained the better appearance, binding, juiciness, and overall acceptability. The obtained findings were in good agreement with the observations that the antioxidant properties of chitosan minimize lipid oxidation and improve color stability (Suman *et al.*, 2007; Lynch *et al.*, 2008) and the sensory quality of meat products (Georgantelis *et al.*, 2007; Soultos *et al.*, 2008).



Fig. 2. Scanning Electron Micrographs of cooked camel luncheon sausage. A, Control (x500), B control (x2000); C with chitosan (x500), D with chitosan (x2000).

The surface ultrastructure of raw camel meat batter showed even dispersion of fat globules of variable shape and size in a dense salt soluble protein matrix with the presence of several insoluble connective tissue filaments (Fig. 1. A). Some fat globules were covered with a dense interfacial protein film, while others were just entrapped in the viscous matrix. Several breaks of the protein film surrounding the fat globules and even loss of the continuity of the film were evident (Fig. 1. B). The addition of chitosan significantly altered the ultrastructure of the raw batter where all the fat globules appeared totally covered with a dense protein film and embedded in a more dense protein matrix with the complete absence of cracks in the interfacial protein film (Fig 1. C). Higher magnification demonstrated the presence of a dense film completely entrapped the individual fat globules (Fig. 1. D). The ultrastructure of the cooked emulsion prepared without chitosan revealed permanent entrapping of the fat globules away from each other in the protein matrix. The soluble protein matrix showed multiple large holes and aggregation of large fat globules (Fig. 2. A) with multiple and deep cracks in the interfacial protein films surrounding the fat globules and aggregation of the connective tissue protein (Fig. 2. B). The addition of chitosan resulted in a more compact and dense protein matrix with granulation of the insoluble connective tissue protein (Fig. 2. C). The fat globules appeared completely covered with protein film (Fig. 2 D), with an intact protein matrix. The histological details verified that chitosan improved both the formation and integrity of the interfacial protein films around the fat globules as well as the viscosity of the protein matrix which finally enhanced the stability indexes of camel meat emulsion.

CONCLUSION

The obtained data revealed that the addition of chitosan can improve the sensory parameters of camel luncheon sausage with a distinct antioxidant effect and a pronounced emulsion stabilization. Therefore, it can be applied as a thickener and stabilizer for camel meat emulsion to overcome the problems associated with the high connective tissue content tissue and thermal stability of camel meat and to produce more stable products through 5 months of refrigerated storage. Furthermore, the addition of chitosan can satisfy both the consumers and the producers and can be used to produce a higher-quality camel luncheon sausage.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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