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Modification and simplification of Rongey's method for measurement of meat batter stability

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ABSTRACT

The method of Rongey for assessment and quantification of meat batter/emulsion stability has over the years proven useful to many research and industrial laboratories around the world. Unfortunately, its requirement for specialized glassware and a very large centrifugation unit makes the method inaccessible to many modern laboratories. We have, therefore, modified Rongey's original method by adapting it to present-day commercially-available glassware and centrifugation equipment. This modified method was validated by comparing it to Rongey's method on both high-fat (27%) and low-fat (10%) finely comminuted pork batters, each with and without the addition of salt (1.8%) and sodium phosphates (0.5%). This design provided us with batters ranging in stability from very low to very high, thus allowing us to compare the methods across analytical extremes.

This modified method:

- Utilizes glassware and centrifugation equipment that are commercially-available today.
- Maintains the simplicity and speed of the original method of Rongey.
- Yields results that are comparable to those of Rongey's traditional method.

Specifications table

Subject area:	Food Science
More specific subject area:	Meat processing
Method name:	Simplified Rongey method for measurement of meat batter stability
Name and reference of original method:	E.H. Rongey, A simple objective test for sausage emulsion quality, Proceedings of the Meat Industry Research Conference (1965), 99–106, American Meat Institute Foundation, Arlington, VA.
Resource availability:	N/A

Background

In 1965, E. H. Rongey first described a practical method for the objective assessment and quantification of the stability of meat batters or emulsions [9]. The method is based on measuring the amount of lipid and water released upon cooking of a meat batter/emulsion and relating the results to the relative stability of the batter. It involves placing predetermined amounts (approximately 25 g) of meat batter samples into specially-designed and custom-fabricated centrifuge tubes (described by [13]), hereafter referred to as Wierbicki tubes Fig. 1, followed by heating the samples in a water bath, low-speed centrifugation, and quantification of the

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released aqueous and lipid fractions. The advantage of the test lies in the unique design of the Wierbicki tube, which consists of a narrow, graduated, glass centrifuge tube (the bottom section) with its mouth fused onto the bottom of a wider, conical glass centrifuge tube (the top section). A fritted glass disc is placed inside the tube in order to keep the solid particles of meat batter in the top section of the tube while allowing liquids released during heating and centrifugation to collect in the bottom section. As these liquids collect in the tube's bottom section, the lipid fraction separates from the aqueous fraction and, since the bottom section is graduated, the volumes of both fractions can be measured separately. In order to serve as a suitable divider, the diameter of the fritted glass disc must be intermediate between the inside diameters of the top and bottom sections of the Wierbicki tube. The unique features of the Wierbicki tube thus allow for (1) complete separation and isolation of released liquids (lipid and aqueous) from the rest of the meat batter and (2) quantification of both fractions.

The Rongey method has proven valuable over time for estimating and quantifying the degree of stability of a meat batter or emulsion, not only in various research laboratories [1,5–8,11], but also in industrial product development [2,10,12]. Unfortunately, the unique features of the Wierbicki tube place limitations on many present-day commercial and research laboratories. First, the tube must be custom-made, which necessitates the work of a specialized glassworks shop. Second, the tube is large in size (approximately 230 mm in length for the ones used in our laboratory), and therefore requires a centrifuge with a larger rotational radius than is found in most modern commercially-available laboratory benchtop centrifugation units. While similar methods to quantify meat batter stability that rely on commercially-available centrifuges and centrifuge tubes have been used routinely by research laboratories around the world (e.g., [3,4]), in many of these the separation of released liquids is less effective, and their quantification more complex, less accurate and more time-consuming.

We, therefore, propose a variation of the Rongey methodology which utilizes present-day commercially-available centrifugation instrumentation and glassware, with the objective of making a meat batter stability quantification method that is simple, fast, accurate, and repeatable, more widely accessible to laboratories and research groups worldwide. We hypothesize that our proposed methodology will be equivalent to Rongey's for the estimation of lipid and water released from meat batters.

Method details

Materials

Traditional Rongey method

Glassware: Wierbicki tubes (Fig. 1) custom made in the Glass Shop of Iowa State University's Department of Chemistry, by cutting the bottoms of 32 mm (O.D.) x 28 mm (I.D.) conical borosilicate glass centrifuge tubes and fusing them onto the cut mouths of Pyrex no. 8140 conical borosilicate glass centrifuge tubes (17 mm O.D.) with 0.1 mL graduation intervals (0–12 mL) (Corning Inc., Corning, NY, USA). The length of the tubes is approximately 230 mm. These tubes were themselves slightly modified from the tube first described by Wierbicki et al. [13], in that the bottom section has a tapered, as opposed to rounded, tip — a modification made to adapt them to the centrifuge available in our laboratory, and to facilitate reading volumes < 0.5 mL. Wierbicki et al.'s original tube was also shorter than ours (180 mm vs. 230 mm, respectively), but would still be too long to fit in most modern tabletop centrifugation units.

Sample size: 25.0 ± 1.0 g.

Centrifugation unit: Chicago Surgical & Electrical model 61 centrifuge (Labline Inc., Chicago, IL, USA). Because this centrifuge model was discontinued decades ago, rotational speed was measured using a handheld optical tachometer (model HHT12, Omega Engineering, Inc., Norwalk, CT, USA) rather than its built-in analog tachometer dial, deemed to be inaccurate

Modified method

Glassware: Kimax no. 45165 conical borosilicate glass centrifuge tubes (DWK Life Sciences, Millville, NJ, USA) (Fig. 1); graduation intervals: 0.5 mL (0–10 mL), 1 mL (10–50 mL); dimensions: 28.5 mm (O.D.) x 26 mm (I.D.) x 134 mm (length). These specific tubes were selected because (1) their internal diameter is similar to that of the top section of the Wierbicki tube (which facilitates entry of fritted glass disc and dispensing by syringe barrel), and (2) they begin to taper far enough away from their tip such that the fritted glass disc, when inserted, stops at near the 13-mL graduation mark, thus providing enough empty space below it for accumulation and measurement of released liquids. Prior experience has shown us that a minimum of 10 mL of empty volume below the fritted glass disk should be available to capture the liquids released by highly unstable batters. Tubes that begin to taper too close to the tip lack enough empty space and are, therefore, unsuitable for this method.

Sample size: 15.0 ± 1.0 g. Because this tube is smaller, it cannot accommodate a 25-g sample, like the Wierbicki tube can.

Centrifugation unit: Thermo Scientific Sorvall Legend X1R centrifuge (Thermo Electron LED GmbH, Osterode am Harz, Germany) equipped with a Thermo TX-400 rotor and Thermo 75003655 buckets.

Method validation

Meat and ingredients

Fresh pork meat trimmings of varying fat levels were obtained from the Iowa State University Meats Laboratory (Ames, IA, USA), ground to 4.5 mm, and pre-analyzed for fat and moisture prior to use following AOAC Official Method 2008.06 (Smart 6 moisture analyzer/Oracle fat analyzer, CEM Corporation, Matthews, NC, USA). The fat contents of the trimmings were 3.1% (low fat), 23.9%

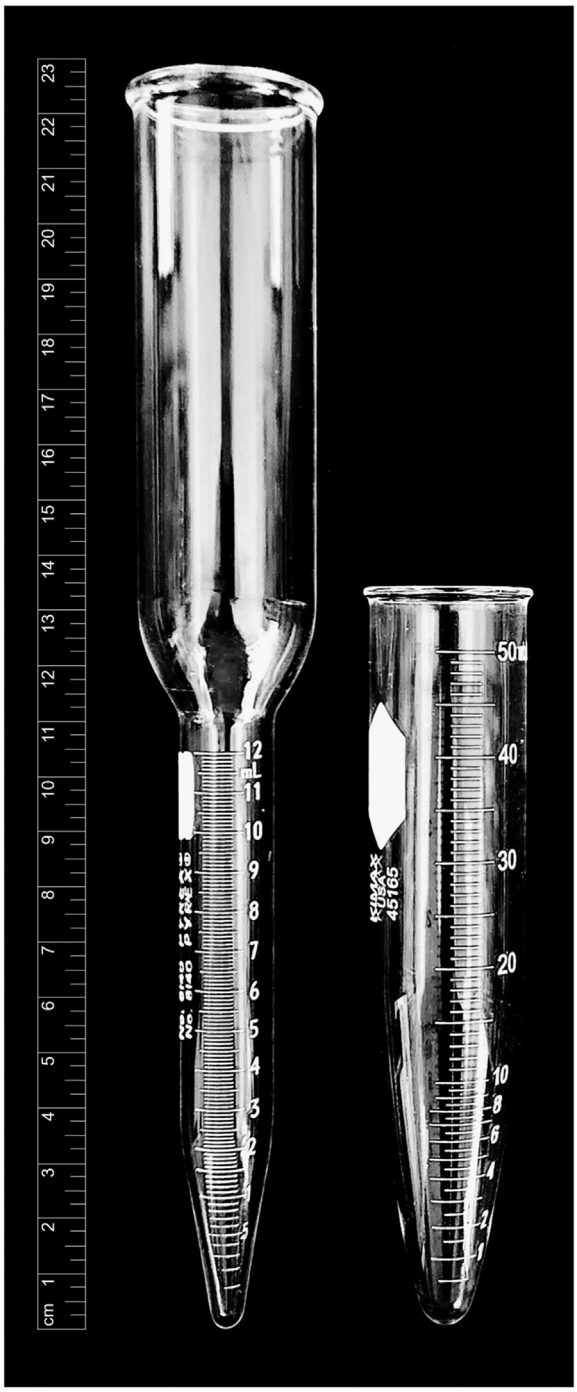

Fig. 1. Wierbicki tube used in Rongey's batter stability method (left) and centrifuge tube used in modified batter stability method (right).

(intermediate fat) and 66.6% (high fat). Salt was obtained from A.C. Legg, Inc. (Calera, AL, USA), and sodium phosphate (Brifisol 512; blend of sodium tripolyphosphate and sodium hexametaphosphate) from BK Giulini GmbH (Ladenburg, Germany).

Meat batter formulation, preparation and analysis

The validation experiment was done in a 2 × 2 × 2 factorial design, with two levels of factor FAT (high fat = 27%; low fat = 10%) and two levels of factor SPH (1.8% salt + 0.5% sodium phosphate; no salt/phosphate), for a total of 4 batters, the stability of which was measured by both methods (factor METHOD). The design of the 4 batter formulations provided batters ranging in stability from

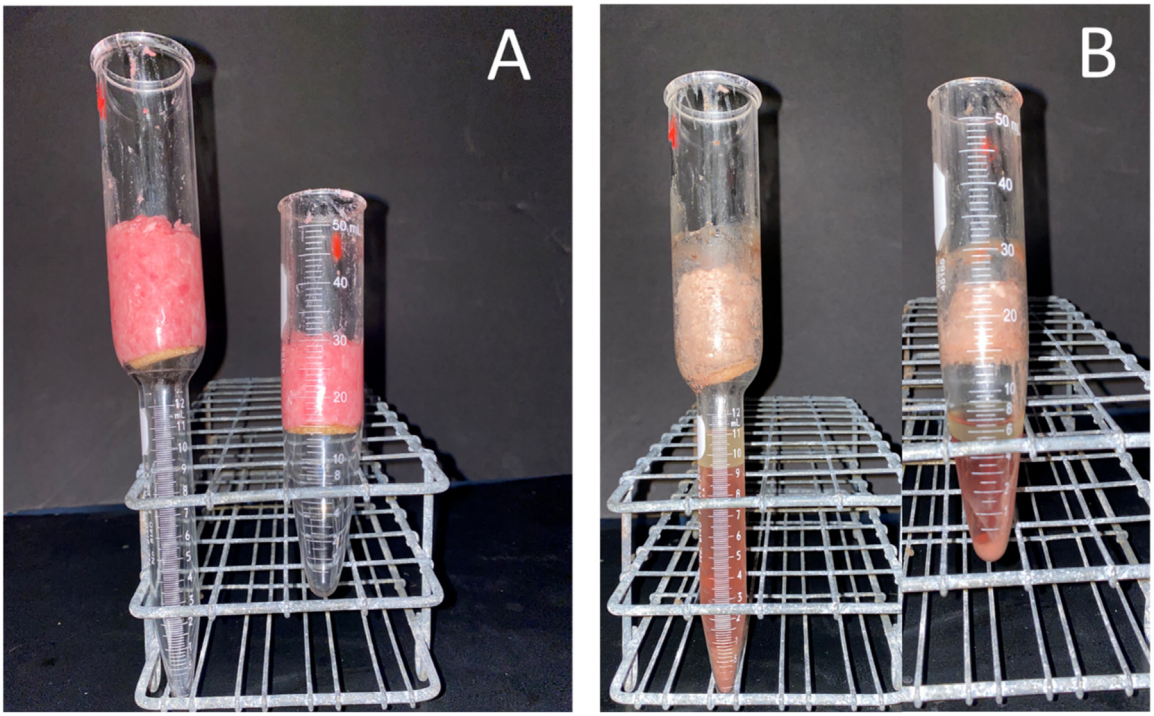

Fig. 2. Tubes filled with meat batter, before heating (A) and after heating and centrifugation (B).

very high to very low. The meat batters were made by combining 2268 g of a blend of pork meat trimmings with 454 g of an ice/water mixture. The blends were formulated to their corresponding target fat content by combining the high-fat trimmings with either the intermediate- or low-fat trimmings. For each batch, the leaner of the two pork trimmings were comminuted with ice, salt and phosphate (if applicable) in a bowl chopper (model 84181D, Hobart Manufacturing Co., Troy, OH, USA; 45.7 cm dia. bowl; 4-knife assembly; 3450 rpm;) for 1 min, after which the fattier trimmings were added and chopping continued to 11.1 ˚C.

Method steps

Portions of each batter (sample sizes described above; exact weights were recorded) were then inserted into 10 of each of the two types of testing tubes, using a 30-mL open-ended syringe barrel (22.5 mm I.D.), being careful to avoid air pockets inside the batter sample (Fig. 2A). A 25-mm fritted glass disc was previously inserted in each tube to separate meat solids from released liquids. The tubes were heated in a water bath at 71 ˚C for 30 min, allowed to cool at room temperature for 3 min and centrifuged at 300 x *g* for 5 min at room temperature (Fig. 2B). Immediately following centrifugation, aqueous (bottom) and lipid (top) layers were read from the graduated section of each tube and released liquids were calculated as follows:

$$\%\ \text{Water separation} = \frac{\text{water volume (mL)}}{\text{sample weight (g)}} \text{ x } 100 \quad (1)$$

$$\%\ \text{Lipid separation} = \frac{\text{lipid volume (mL)}}{\text{sample weight (g)}} \text{ x } 100 \quad (2)$$

Experimental design and statistical analysis

The validation experiment was designed as a randomized complete block (RCBD), with factors FAT and SPH arranged in a split block and factor METHOD arranged in a split plot in relation to FAT and SPH. For each replication, assignment of experimental treatments to batches was done randomly. The experiment was replicated independently three times, with replications as blocks. Data were analyzed as a mixed model using JMP Pro version 16.2.0 (SAS Institute Inc., Cary, NC, USA) with FAT, SPH, METHOD, and their two- and three-way interactions as fixed factors, and replication as random factor. Differences between means were determined using the Tukey-Kramer pairwise comparison method. Significance was established at $P < 0.05$.

Data and discussion

P values of main effects and their interactions are shown in Table 1, and means for released water and lipid values are shown in Table 2. The FAT x SPH interaction effect was significant for released water and lipid, both of which were, expectedly, lower when salt and phosphate were added (Table 2). The significant interaction effect for released lipid, however, was driven by higher values

Table 1
P values[1] of fixed main effects[2] and interactions of liquids released from cooked pork batters following centrifugation.

Dependent variable	Main effects			Interaction effects			
	FAT (*F*)	SPH (*S*)	METHOD (*M*)	*F* × *S*	*F* × *M*	*S* × *M*	*F* × *S* × *M*
Released water, %	**0.026**	**0.003**	**0.002**	**0.020**	0.167	**0.006**	0.353
Released lipid, %	0.050	**0.039**	0.930	**< 0.001**	0.408	0.705	0.357

[1] Statistical significance established at $P < 0.05$ (shown in bold).
[2] FAT: fat level (high; low); SPH: salt & phosphate added (no; yes); METHOD: batter stability method (Rongey; modified).

Table 2
Least squares means[1] for water and lipid released from cooked pork batters after centrifugation, as affected by fat level and addition of salt and phosphate, as measured by two different batter stability analytical methods.

Fat level	Salt/phosphate addition	Released water (%)		Released lipid (%)	
		Rongey	Modified	Rongey	Modified
High	Yes	4.6 (0.19)^{c}	5.8 (0.60)^{c}	0.8 (0.08)^{b}	0.9 (0.10)^{b}
High	No	29.4 (0.46)^{a}	24.6 (0.51)^{ab}	9.6 (0.60)^{a}	8.3 (0.58)^{a}
Low	Yes	6.4 (0.57)^{c}	4.4 (0.31)^{c}	0.5 (0.07)^{b}	0.6 (0.07)^{b}
Low	No	26.2 (0.59)^{a}	20.8 (0.78)^{b}	0.6 (0.04)^{b}	1.1 (0.11)^{b}

$^{a\text{-}c}$Within released liquid type (across method), means with different letters are significantly different (based on 95% Tukey-Kramer confidence intervals).
[1] $n = 30$ (10 tubes/batch x 1 batch/replication x 3 replications); numbers in parentheses indicate standard errors.

in the absence of salt and phosphate in the high-fat treatment only. No differences were observed, regardless of fat level, when salt and phosphate were added, nor in the absence of salt in the low-fat treatment. Regarding released water, the significant FAT x SPH interaction effect was driven by high values in the high-fat treatment and intermediate values in the low-fat treatment, in the absence of salt and phosphate. In the presence of salt and phosphate, released water values were lower and not different from each other, regardless of fat level. The results suggest that, in the absence of salt and phosphate, the high-fat batter was more unstable, whereas in the low-fat batter addition of salt and phosphate resulted in greater ability to hold water, but not lipid. The lack of a significant three-way (FAT x SPH x METHOD) interaction reveals that the batter stability analysis method played no part in these observed effects and that both methods had equivalent performance, regardless of the fat content of the batter.

The SPH x METHOD interaction was significant for released water, but not for released fat (Table 1). In the presence of salt and phosphate, released water, regardless of method, was lower than in their absence. However, in the absence of salt and phosphate, it was higher ($P < 0.05$). for the Rongey method than for the modified method (27.7% v. 22.7%, respectively [$P < 0.05$]; means averaged across FAT). Close examination of treatment means within each fat content (Table 2) reveals that released water in the absence of salt and phosphate was not different between methods at high fat, and lower in the modified method than the Rongey method at low fat. All other interaction effects involving METHOD were not significant, indicating equivalent performance of the two methods under the conditions tested.

Although the number of formulation conditions tested in this study was limited, given that the modifications proposed do not alter the fundamental principle on which Rongey's original method is based, we anticipate this modified method will perform equally well under various other meat batter formulation conditions, such as variations in type of meat or poultry species, intermediate-stability formulations, and the addition of other ingredients, such as hydrocolloids and other binders. These conditions, however, remain to be tested.

Conclusion and significance

The proposed modification of the Rongey methodology yielded results comparable to those of the original method, with the exception of released water in the low-fat, no salt/phosphate treatment, where it yielded slightly lower results. The adaptation of Rongey's method to centrifugation equipment, glassware and supplies that are all commonly used at present, makes this valuable research tool available to the greater meat and food science research community, as well as industrial laboratories worldwide.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sarah E. Bludau: Validation, Investigation, Data curation. **Rachel I. Crowley:** Investigation. **Bailey C. Hauge:** Investigation. **Rodrigo Tarté:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

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