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Appendix A:
Plan to Achieve 6 Repetitions of 5 Treatments (Repeated Twice for Both Liquid and Dry Honey)

Appendix A: Plan to Achieve 6 Repetitions of 5 Treatments (Repeated Twice for Both Liquid and Dry Honey)

	<u>Liquid Honey</u>	<u>Dry Honey</u>	
Block 1:	1, 2, 3	1, 2, 6	where:
Block 2:	1, 2, 5	1, 2, 8	1- Full Fat Control
Block 3:	1, 4, 5	1, 7, 8	2- Fat Reduced Control
Block 4:	2, 3, 4	2, 6, 7	3- 25% Liq. Replace
Block 5:	3, 4, 5	6, 7, 8	4- 35% Liq. Replace
Block 6:	1, 2, 4	1, 2, 7	5- 45% Liq. Replace
Block 7:	1, 3, 4	1, 6, 7	6- 25% Dry Replace
Block 8:	1, 3, 5	1, 6, 8	7- 35% Dry Replace
Block 9:	2, 3, 5	2, 6, 8	8- 45% Dry Replace
Block 10:	2, 4, 5	2, 7, 8	

Appendix B:
Formulas for a Full Fat Muffin Control and a Fat Reduced Muffin Control

Appendix B: Formulas for a Full Fat Muffin Control and a Fat Reduced Muffin Control

Ingredients:	FULL FAT CONTROL *	FAT FREE CONTROL *
Flour, Southern Biscuit	337.5 g	337.5 g
Sugar, Granulated	150.0 g	150.0 g
Baking Soda	4.5 g	4.5 g
Baking Powder	17.1 g	17.1 g
Salt	3.75 g	3.75 g
Eggs	64.5 g (whites only)	64.5 g (whites only)
Nonfat Buttermilk	160.1 g	160.1 g
Skim Milk	177.0 g	177.0 g
Water:	-	-
Vegetable Oil	88.5 g	-
Vanilla	6.9 g	6.9 g
Fat Substitute (Methocel)	-	1.5 g
Enzymes- fungal amylase	-	0.0045 g
- bacterial amylase	-	0.0045 g
emulsifiers :Datem	-	1.8 g

* Mason et al., 1996

Appendix C:

**Formulas for a 25%, 35%, and 45% Replacement of Either Liquid or Dry Honey
for Sucrose**

Appendix C: Formulas for a 25%, 35%, and 45% Replacement of Either Liquid or Dry Honey for Sucrose

LIQUID HONEY:

Ingredients:	25% liq	35% liq	45% liq
Flour, Southern Biscuit	337.5 g	337.5g	337.5 g
Sugar, Granulated	112.5 g	105.0 g	82.5 g
Liquid Clover Honey	45.0 g	63 g	81.0 g
Dry Honey	-	-	-
Baking Soda	4.5 g	4.5g	4.5 g
Baking Powder	17.1 g	17.1g	17.1 g
Salt	3.8 g	3.8 g	3.8 g
Eggs	64.5 g (white only)	64.5 g (whites only)	64.5 g (whites only)
Nonfat Buttermilk	156.3g	155.6 g	154.1 g
Skim Milk	173.3 g	172.5 g	171.0 g
Water:	-	-	-
Vegetable Oil	-	-	-
Vanilla	6.9 g	6.9 g	6.9 g
Fat Substitute (Methocel)	1.5 g	1.5 g	1.5 g
Enzymes- fungal amylase	0.0045 g	0.0045 g	0.0045 g
- bacterial amylase	0.0045 g	0.0045 g	0.0045 g
emulsifiers :Datem	1.8 g	1.8 g	1.8 g

Dry Honey

Ingredients:	25% dry	35% dry	45% dry
Flour, Southern Biscuit	337.5 g	337.5g	337.5 g
Sugar, Granulated	112.5 g	97.5g	82.5 g
Liquid Clover Honey	-	-	-
Dry Honey	37.5 g	52.5 g	67.5 g
Baking Soda	4.5 g	4.5g	4.5 g
Baking Powder	17.1 g	17.1 g	17.1 g
Salt	3.8 g	3.8 g	3.8 g
Eggs	64.5 g (white only)	64.5 g (whites only)	64.5 g (whites only)
Nonfat Buttermilk	156.3 g	155.6 g	154.1 g
Skim Milk	173.3 g	172.5 g	171.0 g
Water:	37.5 g	52.5 g	67.5 g
Vegetable Oil	-	-	-
Vanilla	6.9 g	6.9 g	6.9 g
Fat Substitute (Methocel)	1.5 g	1.5 g	1.5g
Enzymes- fungal amylase	0.0045 g	0.0045 g	0.0045 g
- bacterial amylase	0.0045 g	0.0045 g	0.0045 g
emulsifier:Datem	1.8 g	1.8 g	1.8 g

Appendix D:
Determination of Moisture Content

Appendix D: Determination of Moisture Content

1. Turn on the Brabender Moisture Tester Model SAS (Hackensack, NJ) machine one hour prior to analysis by pressing the switch up on the left hand side of the unit and on the bottom right front of the machine. This is done to enable the machine to reach the desired temperature of 130°C.
2. Three Teflon lined metal pans were weighed individually (1 for each treatment in any given block) on an OHAUS balance Model CT 1200 (Florham Park, NJ). The balance was tared and the weights of the empty pans recorded.
3. The interior of the muffin was torn into small pieces and ten grams of sample were weighed into the tared pan.
4. After opening the door on the Brabender by twisting the knob on the oven door, the pan and sample were placed in the oven. Additional samples were placed in the sample holders by twisting the black knob located on the left side of the unit.
5. The samples were left in the machine for 1 hour.
6. To determine when equilibrium was attained, quarterly hour measurements were taken by:
 - a. Turning off the fan on the front of the unit.
 - b. Opening up the door of the square unit by lifting up on the latch located on the right hand side.

- c. Placing a 2 g weight on the center, beneath the samples and closing the door.
- d. Lifting the handle up on the left hand side of the unit. A scale should appear in the circular window on the right-front of the machine.
- e. Waiting for the scale to stabilize, record the value after adding 30 to this value.
- f. The sample reached equilibrium when two quarterly readings had not differed by ± 2 .

7. After 1 hour, a 3 gram weight was placed on the pedestal of the balance located on the front of the Brabender machine. The pan containing the dried sample was weighed in the machine by depressing a lever (located on the left of the machine) downward. A switch located on the right of the machine was switched to the off position so that the weight of the pan and sample could be read. The moisture content of the sample was calculated with the following equation:

$$\% \text{ moisture} = (\text{number read on the front of the machine}) + 30$$

Appendix E:
Determination of Water Activity

Appendix E: Determination of Water Activity

1. Turn on the power switch 30 minutes prior to analysis to allow the Decagon Aqua Lab CX-2 Water Activity Meter (Pullman, WA) to warm-up.
2. Fill a plastic disposable cup (40mm x 13 mm) half full with distilled water until it is half full.
3. Place distilled water sample in sample drawer by turning the knob to “load” and pulling out the sample drawer.
4. Close the drawer and turn the knob counterclockwise to “read”. Upon completion, the unit will beep and the LCD display will blink. The display should read 1.000 ± 0.002 . If the unit does not display this value, then it should be recalibrated by turning the calibration screw (located on the back of the machine) accordingly until LCD panel reads 1.000.
5. Remove crumb from the center of the muffin and press into the bottom of the disposable water activity cup, such that the cup is half full.
6. Remove the distilled water sample from the sample drawer, place the muffin sample into the sample drawer, close the drawer, and turn the knob to “read”.
7. Upon completion, the unit will beep and the LCD display will blink. Record this value, and turn the knob to open to remove the sample.
8. Load the next sample.

Appendix F:
Standardization of the Hunter Colorimeter

Appendix F: Standardization of the Hunter Colorimeter

1. Switch the Hunter Lab Optical Sensor 45/0 D25-PC2 (HunterLab, Reston, VA) to “operate” (from standby) and remove the white uncalibrated tile from the specimen port.
2. Place the black standard glass tile on the specimen port, shiny side toward the port. Depress “F1” (bottom of the scale is standardized (“zeroed”)).
3. Remove the black tile and place the white tile in the specimen port. Depress “F1” (top of the scale is standardized (“zeroed”)).
4. Depress “F1” to place the measurement mode into delta E values of L, a, and b.
5. Depress “F8”, “#1”, and “F2” to place the standard values into L, a, and b.
6. Put first specimen into specimen port and press “F1” to measure.
7. Depress “Ctrl” and “Print Screen” simultaneously to print results.

Appendix G:
Operation of the Differential Scanning Calorimeter (DSC)

Appendix G: Operation of the Differential Scanning Calorimeter (DSC).

1. Turn on the cooler/heater circulating system (Fisher Scientific Model 9105, Pittsburgh, PA) one hour prior to use.
2. Turn on the Perkin Elmer Differential Scanning Calorimeter DSC7 #5021501 (Norwalk, CT).
3. Turn on the Perkin Elmer Thermal Analysis Controller DSC7/DX (Norwalk, CT).
4. Turn on the Digital DECpc Lpv+ 433 dx personal computer and Hewlett Packard (San Diego, CA) 7475A Plotter #61039.
5. Place a sheet of non-glossy paper into the plotter.
6. Upon completion of the “booting up”, select “Run System”.
7. Next, “Run 7 Series Unix System” was selected followed by “*2” (to perform multiple tasks during the operation of the unit).
8. “F2”, “Set Up and Run”, press Enter.
9. “F2” , Recall Method”, press Enter.
10. Upon calibration of the machine (first trial of the particular testing day), highlight the most recent indium file, press Enter.
11. Press “F3”, “Modify Parameters”, to modify and rename the file. When complete, press “F1” to exit.
12. Press “F11”, “Go to Temp” to set the starting temperature of the run.
*for the indium calibration run, the starting temperature was 50°C.
*for the muffin run, the starting temperature was 17°C.
13. Wait for the control light on the DSC 7 Controller unit to light up, and the milliwatt signal (on the upper left hand corner of the computer screen) to stabilize.
14. Load the sample into the machine by lifting the hinge and turning it to the right, exposing the load cells. Place the sample to be analyzed in the left receptacle and the reference (blank dish) in the right receptacle. Close the lid and press the hinge down.

15. Wait for the control light to come back on and the milliwatt reading to restabilize, then press “F8”, “Start Run”.
16. Press “Enter” upon completion of the test, then “F5” to “Optimize” the data.

Press “F2” to “Rescale” the graph generated (temperature, x-axis vs. heat flow, y-axis).

Press “F3”, to “Autoscale”.

Press “F1” once to “Exit”.

Press “F4” to determine the “Slope” of the curve.

Press “F3” to “Align” the points.

Press “F1” twice to “Exit”.

Press “F6” to “Select Calculations”.

Press “F2” to determines the “Peak”.

Press “F4” to “Include Onset”. At this time use the left and right buttons on the mouse to define the start and finish of the peak.

Press “F7” to “Autocalculate”

Press “Print Screen” to print the results of the run.

When the monitor reads “Plot is queued”, then press “Enter”.
17. If the onset temperature of the standard is $156.6 \pm 0.2^{\circ}\text{C}$, the instrument is calibrated. If not, calibration of the instrument is necessary.
18. If the onset temperature is within limits, press “F11” and enter 17.0°C , so the unit can be cooling down to 17.0°C , to run the samples. Press “F1” twice to exit.
19. Remove the Indium standard only.
20. Press “F2”, “Set Up and Run”, and then “F3”, Modify Parameters. Make the necessary changes. Press Return to enter the new changes. Press “F1”, “Exit” when changes are complete.

Wait for the milliwatt reading to stabilize and for the control light to come on before loading a sample. See Sample Preparation.

21. After the sample has been prepared, load the sample into the left side of the sample holder after the control light is lit and the milliwatt reading has stabilized.
22. Wait for the control light to come back on and for the milliwatt reading to stabilize. When this occurs, press “F8”, “Start Run”.
23. At the end of the run:
Press ENTER.

Press “F5”, “Optimize Data”.

Press “F2”, “Rescale”.

Press “F4”, “Rescale X axis”.

Type in “30”, “Enter”, “110”, “Enter”.

Press “F2”, “Rescale”.

Press “F1”, “Exit”.

Press “F3”, “Shift y”.

Press “F2”, “Shift y”.

Type in “0.4”, “Enter”.

Press “F1”, “Exit”.

Press “F2”, “Rescale”.

Press “F5”, “Y Limits”.

Type “0”, “Enter” then “1”, “Enter”.

Press “F1”, “Exit”.

Press “F4”, “Slope”.

Press “F3”, “Align Endpoints”.

Press “F1”, twice.

Press “F6”, “Select Calc”.

Press “F2”, “Peak”.

Press “F4”, “Include Onset”.

Use the mouse to define the beginning and end of the peak.

Press “F2”, twice.

Press the “Print Screen” button, then “Enter” on the plotter.

24. Run remaining samples by following steps 18-23.

Sample Preparation:

1. Calibrate the microbalance (Perkin Elmer, Norwalk, CT). Twist the gray knob on the left hand side to lower the holders.
2. Press autotare. Zeroes should be displayed.
3. Twist the gray knob until the holders support the discs.
4. Place a 20 mg weight on the balance, release the supports and make sure the balance displays 20.0. Remove the weight and raise the support. If it does not, the machine needs to be calibrated. Follow in calibration procedure in the manual.
5. Obtain the top and bottom of the stainless steel large volume pan (Part # 0319-0218, Perkin Elmer, Norwalk, CT) and place the sample pan on the bottom portion of the scale. Lower the supports and press autotare. Raise the supports and remove the pan.
6. Remove a small portion of the crumb from the center of the muffin, roll it into a small ball and place it in the pan. Weigh to a final weight of 40.0 ± 0.3 mg.
7. Place an “O-ring” in the top portion of the pan and place on top of the bottom of the pan.

8. Place the sample pan with lid and “O-ring” on top of the sample holder and place in the crimper (Perkin-Elmer, Norwalk, CT). Crimp by pressing the handle down.

Calibration of the DSC:

1. Press CONTROL+S (Shutdown).
2. Press “y”, “Yes”.
3. Wait approximately 1 minute.
4. At the main menu, highlight “Calibrate an Analyzer”. Press “Enter”.
5. Press “F2”, “Calibrate Temp/Area”.
6. The screen will display several parameters. In the bottom left and corner where it reads “Measured Onset Temperature and Measured J/g”, enter in those values obtained from the plot of the last run Indium standard.
7. Press “F7”, “Begin Cal”.
8. Press “Ctrl Shutdown”, then “Y”.
9. From the main menu, highlight “Run Series UNIX System”. Press ENTER.
10. Select “*2”, ENTER.
11. Press “F2”, “Recall Method” and select and run the indium standard to see if the unit is calibrated.

Shutdown Procedure:

1. Press CTRL Shutdown (the “zero” button). Answer “y” for yes to shutdown all processes. When the monitor reads, “Are you sure?”, type in “yes”, Press “Enter”.
2. Wait one minute.
3. Highlight “Shutdown the Unix Operating System”.

4. When the monitor reads “System is now shutting down. Safe to turn power off, turn off the TAC&/DSC-7 Unit, DSC-7 Controller, computer, monitor, and plotter.
5. Remove the sample and reference pans.
6. Turn off the chiller/circulator.

Appendix H:
Determination of Crumb Firmness

Appendix H: Determination of Crumb Firmness

1. Turn on the Instron Model 1011 (Canton, MA) and press the “GPIB” button, so then metered light is on, followed by the computer, monitor, and printer.
2. Highlight “Test a sample”, press “Enter”.
3. Enter your last name beside “Operator”.
Enter a sample id for your sample (up to 6 letters).
The method should state (Compressive) and the method number is 06.
4. Press “F10”, “Continue”.
5. The menu: “Start specimen: 1” will appear.
Enter in the sample number (ex. 1A1), diameter, and the lens gauge (25% of diameter).
Press “F10”, “Continue”.
6. Remove the top (i.e. crust) off the muffin by making a horizontal incision in order to expose the flat crumb surface.
7. Place the sample under the compression probe. Check the platen distance, which should be 5 mm in this method. If this is not the distance, turn off the GPIB button and use the up and down buttons on the Instron to correct the distance.
Turn the GPIB button back on.
8. Press “F10”, “Continue”.
Press “F10”, “OK”.
Press Enter. This will begin the acquisition.
9. When the compression is complete, the unit will sound a beep.
Press “Enter”. This will return the crosshead. Remove the sample.
Press “F10”, “Continue”.
10. Follow steps 4-9 to analyze the remaining samples.
11. After all of the samples have been tested, press ESC. This automatically prints the data to the printer. The main menu will appear on the screen.
12. Highlight “Quit”, and press “Enter” to exit to DOS.

13. Turn off the computer, monitor, printer, and the Instron.

14. Clear the compression probe and base.

Appendix I:
Determination of Volume

Appendix I: Determination of Volume

1. The muffins were cooled one hour prior to obtaining the measurements.
2. Two muffins from each batch were randomly chosen.
3. A dial caliper was used to measure the height and diameter of the muffin, such that:
 - * Height was determined by inserting the probe into the highest point of the muffin until it touched the base of the muffin.
 - * Diameter was assessed by placing the muffin in between the calipers.
4. After the average of the two muffins were determined for height and diameter, the values obtained were incorporated into the following formula:

$$\text{Volume} = (22/7)r^2h$$

Appendix J:
Approval of Research Involving Human Subjects

Appendix K:
Sensory Panel Informed Consent Form

Appendix K: Sensory Panel Informed Consent Form

Virginia Polytechnic Institute and State University Informed Consent for Participation in Sensory Evaluation.

Title of Project: “The Effect of a Liquid or Dry Honey Replacement for Sugar on the Baking Qualities and Freeze Thaw Stability of Fat Reduced Muffins.”

Principal Investigator: Matthew Strait

I. PURPOSE OF PROJECT:

You have volunteered to participate in a sensory evaluation panel that involves assessing various sensory characteristics (developed by you, the panelists) pertaining to multiple formulations of a reduced fat muffin. Establishing such information will be correlated with physical measurements to ascertain specifically how honey in either a liquid or dry state combined with a fat replacer, fungal and bacterial amylases and an emulsifier system developed previously, affects given parameters. This information will also be compared with values obtained with a full fat muffin (containing none of the above ingredients) on the basis of the same characteristics.

II. PROCEDURES:

There will be a total of 23 sessions, with 2 sessions being held per week for 10 weeks. Each session should take no longer than 15 minutes, except for the 3 training sessions that should last approximately 45 minutes. At each session, you will be presented with 3 muffins. As a panelist, attendance is critical. If you can not attend a particular session at the designated time, please let me know so that arrangements can be made. Should you find a sample offensive or unpalatable you may choose to spit it out and continue to the next sample. Certain individuals may be sensitive to some foods such as milk, eggs, wheat gluten, artificial sweeteners, and etc. If you are aware of any food or drug allergies, please list them on the following page.

III. BENEFITS/RISKS OF THE PROJECT:

Your participation in the project will provide useful information that can be used in further development of this product. All food additives have been approved by the Food and Drug Administration, so there are no risks involved provided you do not have any unknown allergies. You may receive the results or summary of the panel when the project is completed.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY:

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. FREEDOM TO WITHDRAW:

It is essential to sensory evaluation projects that you complete each session. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VI. APPROVAL OF RESEARCH:

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VII. SUBJECT’S RESPONSIBILITIES:

I know of no reason I cannot participate in this study which will require meeting twice per week for 10 weeks for approximately 15 minutes with the exception of 3 training sessions lasting approximately 45 minutes.

X _____
SIGNATURE

Please provide your address, e-mail address, and phone number so I can reach you in case of an emergency or if the schedule should change for some unknown reason

address: _____

e-mail: _____

phone: _____

Please list any allergies, medications, or any other pertinent information:

VIII. SUBJECT’S PERMISSION:

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I can not participate in this study which will require meeting twice per week for 10 weeks for approximately 15 minutes with the exception of 3 training sessions lasting approximately 45 minutes.

X _____
SIGNATURE DATE