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Tom Hammett, PhD
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Dear Dr. Hammett,

Please find detailed in this report the results of the evaluations performed on samples of tree syrups supplied to our laboratory as well as summaries of the investigative research for future project steps.

I. Current Elements
   a. Internal Laboratory Analysis
      i. pH
      ii. Water Activity
      iii. Brix
   b. Comparison to Other Known Syrup Products
   c. Sensory Evaluation
      i. Triangle Test
      ii. Acceptance Test
II. Future Elements
   a. External Laboratory Analysis
      i. Nutrition
      ii. Identification of pectin-like component
   b. Sensory Evaluation
      i. Descriptive Analysis

Thank you for choosing to partner with the Department of Food Science and Technology at Virginia Tech.

Best regards,

Melissa S. Wright, M.S.
Director, Food Producer Technical Assistance Network

I. Current Elements

a. Internal Laboratory Analysis

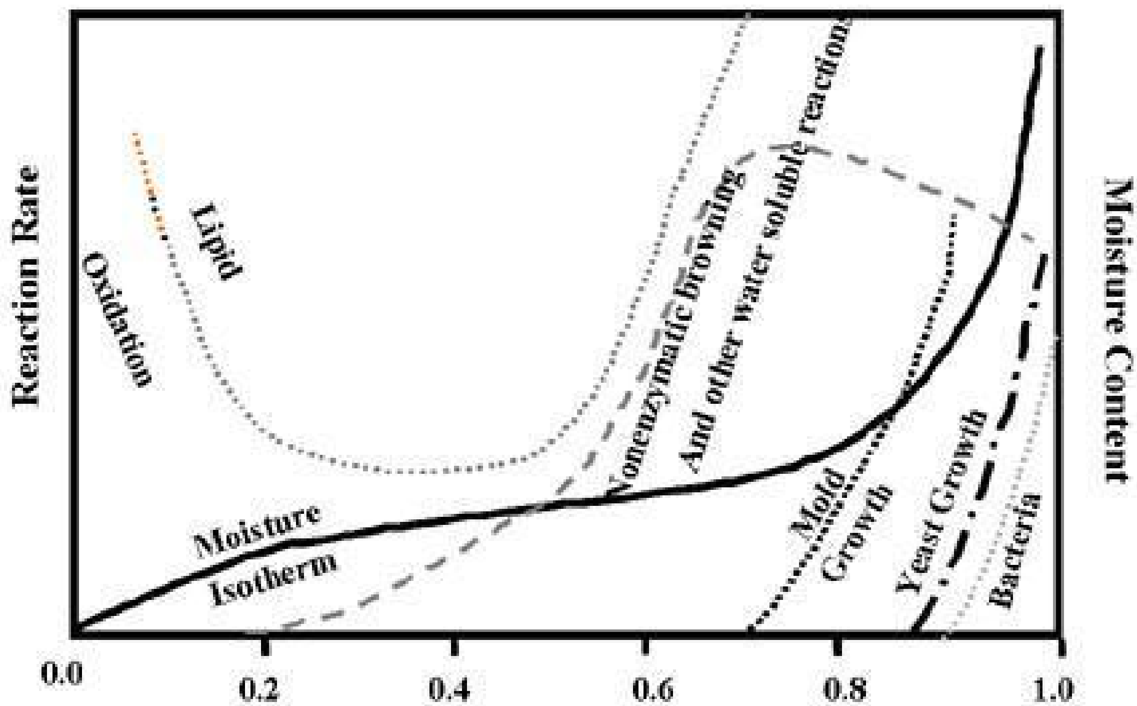
The following samples were received by the laboratory in November 2022 and February 2023 and aliquots were analyzed as detailed below.

	pH - ATC (SU)	Brix - TC (degrees)	Water Activity (a_w)
Black walnut syrup - Tonolway Farm	5.14	69.3	0.8176
Black walnut syrup - unknown origin (1)	5.23	67.6	0.8304
Black walnut syrup - unknown origin (2)	5.14	67.8	0.8051
Black walnut syrup - Mongold's	5.24	64.4	0.8609
Black walnut syrup - unknown origin (3)	5.18	67.4	0.8254
Sycamore syrup - McCoy's (1)	3.09	70.7	0.7301
Sycamore syrup - McCoy's (1)	3.54	70.2	0.7357

The term pH refers to the acidity of a product. In general, the pH of a product will determine which microorganisms are capable of growing in it. Most microorganisms are able to survive and grow in pH environments between 4.6 and 9. Most food items are naturally acidic, meaning their pH value is less than 7.0. As the pH value decreases (become more acidic), microorganisms have a more difficult time surviving and growing. Therefore, the acidity of a food product is often used as a means of preservation and a way to keep food safe for consumption. In general, pathogens of concern (those causing illness) will not grow in products with **pH values below 4.2.**

Water activity refers to the amount of water available in a food product for use by microorganisms to grow. In food systems water can either be bound by ingredients such as salt or sugar, removed through heat (baking, dehydrating, freeze drying, etc.) or be free and available. Water activity is measured using a meter that uses the chilled-mirror dew point technique to measure the water activity of a sample. In an instrument that uses the dew point technique, the sample is equilibrated with the headspace of a sealed chamber that contains a mirror and a means of detecting condensation on the mirror. At equilibrium, the relative humidity of the air in the chamber is the same as the water activity of the sample. In the meter, the mirror temperature is controlled by a thermoelectric cooler. Detection of the exact point at which condensation first appears on the mirror is observed with a photoelectric cell. A beam of light is directed onto the mirror and reflected into a photo detector cell. The photo detector senses the change in reflectance when condensation occurs on the mirror. A thermocouple attached to the mirror then records the temperature at which condensation occurs.

On the following page is a graph that shows the relationship between chemical and microbiological activity and water activity:



Water activity can help predict chemical stability of products. A product with relatively high water activity (above 0.65) allows for both microbial growth (if not eliminated through the heat process and if not prevented from re-contaminating the finished product during filling) and non-enzymatic browning.

Degrees Brix, as measured by a refractometer, has been provided for a measurable target for syrup consistency since water activity meters are far more expensive. Water activity and Degrees Brix measurements do not have a direct relationship unless in a simple solution. Degrees Brix, however, is a direct measurement for present soluble sweetener solids. Many syrups have a Degrees Brix measurement of at least 65 for enhanced stability. With at least this percent soluble sweetener solids, the water activity would be greatly reduced.

All of the products meet the criteria to be classified as non-TCS (not requiring **t**ime/**t**emperature **c**ontrol for **s**afety) foods. The products do not need refrigeration for safety nor does time need to be used as a public health control. The criteria used for this determination was extracted from material published by the FDA and the Institute of Food Technologists (IFT) entitled "Framework developed to determine whether food needs time/temperature control for safety" in Comprehensive Reviews in Food Science and Food Safety (Volume 2, Chapter 8, published in 2003) (Outlined in Figure 1). Under these guidelines, this product will not require refrigeration for safety. A copy of the guidelines used for referral can be found at the following link:

Shttps://www.fda.gov/files/food/published/Evaluation-and-Definition-of-Potentially-Hazardous-Foods.pdf

b. *Comparison to Other Known Syrup Products*

There are two major tree syrups commercially-produced and available for purchase to the general public: maple and birch. Black walnut (*Juglans nigra*) is a relatively recent addition to the growing list of other trees which can be tapped for their sap.

Black walnut trees grow best in well-drained bottoms of the Appalachian region, which comprises most of West Virginia and some of southwestern Virginia. If the collection and processing of other tree saps into syrup, including black walnut, are able to be commercialized, this could be an economic success for growers in these economically-depressed regions of the country.

One of the major limitations to commercialization of black walnut syrup is consumer perception. The general public are used to maple syrup as well as table syrups which they mischaracterize as maple syrup.

c. *Sensory Evaluation*

The objective of the experiment was to determine whether black walnut syrup is similar enough to maple syrup to be a good replacement and if it will be preferred over maple syrup. A triangle test was conducted to determine if there was a significant difference in sensory qualities for maple syrup and black walnut syrup. Then, both syrups were sampled individually and rated on a numerical scale based on their overall liking to establish the acceptance.

i. <u>Triangle Test</u>

Panelists were provided with three samples, two of which are the same and they were asked to identify the different sample. The chance of guessing the correct sample was 1/3. Of the 65 panelists, 33 correctly identified the different syrup in their sample sets. Based on statistical calculations, the black walnut syrup and maple syrup truly have significant taste differences. Where $p<0.5$, the statistical result is significant. In this test, $p=0.0027$.

ii. <u>Acceptance Test</u>

Panelists were provided with two unidentified samples (one maple syrup, one black walnut syrup) and asked to score the sample on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely. A mean liking score of 7 or higher can indicate highly acceptable sensory qualities and be used to formulate consumer and producer acceptance limits for a saleable product.

For overall liking, maple syrup was only slightly more liked than black walnut syrup. The mean rating for maple syrup was 6.43 and the mean rating for black walnut was 6.18. Standard deviation for maple syrup was 1.72 and for black walnut it was 1.84. Based on statistical calculations, the variance in likeability of black walnut syrup and maple syrup is insignificant. Where $p>0.5$, the statistical result is insignificant. In this test, $p=0.392$.

II. Future Elements

a. External Laboratory Analysis

i. Nutrition

The nutritional values of black walnut syrup, including calories, sugars and available nutrients, are largely unknown. Laboratory analysis of all samples currently in our possession will take place this year. While the nutritional values are not likely to be a deciding factor for consumers, offering the product for retail and wholesale requires the display of a nutrition facts panel. To our knowledge, these values have not yet been determined.

ii. Identification of pectin-like component

One of the components of black walnut syrup not present in maple and birch syrups is a semi-solid gelatinous mass that coagulates as the product is concentrated. For lack of a better term, it is currently being referred to as pectin. Identification of this component through various analytical methods can allow for the development of processes to inhibit or remove this component and make processing for retail and/or wholesale a more efficient process.

b. Sensory Evaluation

i. Descriptive Analysis

The process of descriptive analysis creates a lexicon for a food product that can be used to create quality standards and boost marketing campaigns as well as identify potential faults that processors may want to avoid. This is done by gathering a diverse panel of interested individuals who meet several times to taste a variety of samples and express their sensory experiences in words. The sensory scientist leading the panel may help guide the panelists in finding the right words and then combines similar terms until a finite list of descriptive words that represent that particular food product or ingredient have been amassed. This process has been performed several times by the sensory evaluation team within our department and the results have been the subject of several posters and peer-reviewed publications.

Figure 1: FDA and IFT framework for determining if time/temperature is required for safety.

2. Framework for determining if time/temperature conrol is required for safety

The food in question may already be held hot or cold for safety reasons. In this case, and if there is no desire for ambient temperature storage, an analysis using this framework is not needed. If the need to control the temperature of the product for safety reasons is unknown, a review of the food, its ingredients, and general methods of preparation should precede the evaluation of the food. If the food, as described, has a substantial and extensive history of safe use without time/temperature control, and there is enough scientific rationale that supports such safe history of use, then the food may continue to be classified as not requiring temperature control for safety, or non-TCS (see also Chapter 3, section 4.2.).

If there is no known history of safe use, proceed with Step 1.

Step 1—Was the food treated to destroy vegetative cells of potential pathogens and packaged to avoid recontamination? If yes, position your product in Table A according to its pH and water activity (a_w). If not, position your product in Table B according to its pH and a_w.

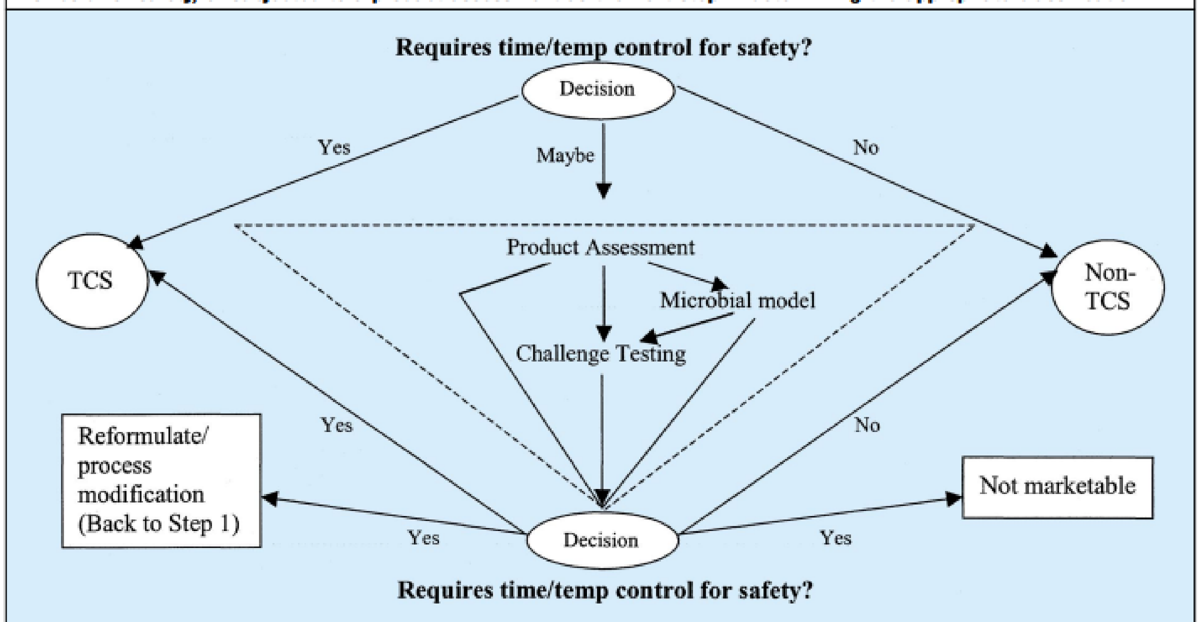
Table A—Control of spores: Product treated to control vegetative cells and protected from recontamination.

Critical a_w values	Critical pH values		
	4.6 or less	> 4.6 to 5.6	> 5.6
0.92 or less	Non-TCS	Non-TCS	Non-TCS
> 0.92 to .95	Non-TCS	Non-TCS	?
> 0.95	Non-TCS	?	?

Table B—Control of vegetative cells and spores: Product not treated or treated but not protected from recontamination

Critical a_w values	Critical pH values			
	< 4.2	4.2 to 4.6	> 4.6 to 5.0	> 5.0
< 0.88	Non-TCS	Non-TCS	Non-TCS	Non-TCS
0.88 to 0.90	Non-TCS	Non-TCS	Non-TCS	?
> 0.90 to .92	Non-TCS	Non-TCS	?	?
> 0.92	Non-TCS	?	?	?

Step 2—If the food is classified as a non-TCS food according to Step 1 above, it may be stored and held safely without regard to time or temperature. If the need for time/temperature control is questionable, the food should be held either hot or cold for safety, or subjected to a product assessment as the next step in determining the appropriate classification.



3. Critique of framework. Application of framework to foods.

The panel's framework on time/temperature control of foods for safety was applied to the following foods as examples. Each step of the framework has been described as it applies to the food under consideration. Most of the data presented were from industry studies submitted to the panel in response to a survey of industry practices to determine whether a food needs time/temperature control (see Appendix B).

3.1. Salad dressings

Product: Viscous, non-particulate[1] pourable salad dressing.

The product is not held hot or cold. The ingredients of the product are eggs, soybean oil, buttermilk, tomato paste, onion, garlic, spices, lemon juice, vinegar (2.5 – 5.4% salt), and potassium sorbate. Microbial hazards: *Clostridium botulinum*. The product is intended to be distributed and stored at ambient temperature for 7 to 9 mo. New product, so there is no history of use.