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Selection and Use of Honey as an Antioxidant in a French Salad Dressing System

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Honeys from various floral sources were analyzed to select for utilization as a sweetener and potential source of antioxidants in the formulation of a salad dressing. On the basis of various indicators of potential antioxidant effectiveness, such as the ORAC (oxygen radical absorbance capacity) assay and identification of phenolic profile carried out by HPLC analysis, clover and blueberry honeys were selected. Dressings were stored under accelerated conditions (37 °C) for six weeks and at ambient (23 °C) and refrigeration (4 °C) temperatures for one year. Salad dressings incorporating honey provided protection against oxidation to a degree similar to that of EDTA, as determined by peroxide value and *p*-anisidine value. This demonstrates the potential for honey to be used as a substitute for EDTA and sweetener (such as HFCS) in commercial salad dressings.

KEYWORDS: Honey; antioxidant; salad dressing; peroxide value; *p*-anisidine value

INTRODUCTION

Lipid oxidation represents a major source of food quality deterioration, resulting in a decrease in the acceptability of food products. The products of oxidation change sensory characteristics, nutritional quality, and safety of food (*1, 2*). Salad dressings consisting of an oil in water emulsion are susceptible to oxidation by transition metals found in the aqueous phase (*3, 4*) and thus contain preservatives such as EDTA, BHA, and BHT to maintain shelf life.

Current consumer trends toward healthy products are inspirational to new product development, including products offering health appeal, such as fat-free or light options (*5*). More consumer attention is also being placed upon nutrient labels, where consumers are seeking information regarding types of preservatives utilized in processed foods (*6*). As mentioned above, salad dressing formulations often contain preservatives such as EDTA, BHA, and BHT to avoid oxidation catalyzed by transition metals found in the aqueous phase. Manufacturers of such products seek to incorporate a healthful twist to some of the traditional favorites and have done so by creating dressings utilizing products such as Benecol and creating all-natural dressings (*7*). Honey is a natural ingredient that enhances sweetness, balances acidity, masks bitterness and saltiness, and adds viscosity (*5*). Honey has been added to salad dressings as a flavoring component; however, studies have not been conducted to test the effectiveness of honey as an antioxidant in this type of emulsion system. Demonstration of honey's capability to serve as a natural antioxidant in salad dressing may give salad dressing manufacturers a natural preservative option.

Honey shows great potential to serve as an antioxidant in an emulsion system. Honey has been incorporated into meat matrices to inhibit lipid oxidation (*8, 9*) as well as to prevent browning reactions in fruits and vegetables (*10*−*12*). Polyphenols, including flavonoids and phenolic acids, are found in honey, and these compounds act as free radical scavengers, peroxy radical scavengers, and as metal chelators (*13, 14*). The antioxidant capacity of a number of honeys has been determined (*15, 16*) and found to be significantly correlated to phenolic content ($R^2 = 0.9497$, $p < 0.0001$).

Formulation of a salad dressing incorporating honey requires careful thought as to the type of honey that would be most beneficial and practical. Honeys from different floral sources vary in color, flavor, availability for commercial purposes, phenolic profile, and antioxidant capacity. The appropriate honey will need to be moderate in flavor and light in color to minimize changes to the appearance and flavor of the dressing. Additionally, stability of salad dressing emulsions may be impacted by diastase enzyme activity of honey that hydrolyzes starch; thus, considerations must be made for the diastase activity of honey. Phenolic profiles generated by HPLC are a useful tool for honey selection. The distribution of phenolic acids and flavonoids, as determined by HPLC, may help characterize what type of antioxidant protection will be provided to a food system.

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Antioxidant capacity, as measured by the ORAC assay, helps predict the degree of protection honey may provide in a food system.

The objective of this study was to select honeys to utilize in a salad dressing formulation, test the stability of the formulation, and test oxidation of the salad dressing oil during a one-year storage period. Honey was expected to protect against oxidation of salad dressing oils when used as a replacement for EDTA and when compared to a dressing formulated with a sugar analogue.

MATERIALS AND METHODS

Blueberry and clover honeys were purchased from McLure's Honey & Maple Products (Littleton, NH). Avocado honey was purchased from Miller's Honey (Colton, CA). Soybean honey was obtained from the University of Illinois Beekeeping Facility (Urbana, IL). All other honeys tested were generously donated by the National Honey Board. These honeys are vended as monofloral, meaning that the honey must derive at least 51% of the constituent nectar or 45% of contaminant pollen from a single floral source (*17*). The honeys may contain nectars from more than one source, but the nominate floral type predominates.

Salad dressing ingredients, excluding honey, fructose, and glucose, were generously donated by Tate and Lyle (Decatur, IL). Food grade glucose and fructose were purchased from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) was purchased from Wako Chemicals, Inc. (Richmond, VA) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) from Fisher Scientific (Pittsburgh, PA). Fluorescein disodium was obtained from Aldrich (Milwaukee, WI). The phenolic standard pinocembrin was purchased from Indofine Chemical Company (Somerville, NJ), and all other standards were purchased from Sigma Chemical (St. Louis, MO). Soluble starch and potassium iodate were purchased from Sigma Chemical (St. Louis, MO). Sodium acetate was purchased from Fisher Scientific (Pittsburgh, PA), and iodine was obtained from EM Industries, Inc. (Gibbstown, NJ).

Procedures for fractionation of honey were adapted from Ferreres and others (*18*) using 25−50 g of honey, as described by Gheldof and others (*19*). Honey (25−50 g) was dissolved in 250 mL of acidified water (pH 2.1) and filtered through a column of Amberlite XAD-2 resin. Sugars and polar compounds were eluted with acidified water (350 mL); the column was washed with 300 mL of neutral water, and phenolic compounds were recovered with methanol (600 mL). Methanol extract was concentrated under vacuum at 40 °C and suspended in water (5 mL). This solution was extracted 3 times with diethyl ether (5 mL) to further purify the flavonoids (*18*). The ether extract was concentrated and dissolved in methanol (0.25 mL) and stored at −80 °C until analyzed. HPLC was performed using a 150 mm × 3.9 mm i.d., 5 μm XTerra RP18 column (Waters Corporation, Milford, MA) with diode array detection (DAD) at 285 and 340 nm. Identification of phenolic compounds in honey was done by comparing retention times and spectral characteristics of unknown analytes to known standards, followed by quantification using the appropriate standard curves. Identified peaks were confirmed using HPLC/MS.

ORAC assay was performed using a modified procedure of Cao and others (*20*) and Ou and others (*21, 22*) as described by Gheldof and others (*19*). A free radical generator, AAPH, generates peroxyl radicals that attack fluorescein, resulting in the loss of fluorescence. This loss of fluorescence can be inhibited by antioxidants and was monitored using a microplate fluorescence reader FL600 (BioTek, Inc., VT) maintained at 37 °C. Fluorescence was measured every 2 min for 50 min or until the last reading declined by >95% of the first reading.

Diastase activity was determined spectrophotometrically using Spectronic Genesys 2 (Garforth, Leeds, UK) via the AOAC method (*23*), developed by Schade and others (*24*) and modified by White (*25, 26*).

French salad dressing formulations were as follows (**Table 1**): (1) corn syrup as the sweetener and EDTA as a preservative; (2) sugar analogue without EDTA; and (3) honey (blueberry and clover, respectively) without EDTA. Dressings were prepared both with starch (also including xanthan gum; 0.9% starch and 0.05% xanthan gum) and without starch (xanthan gum alone, 0.2%). Honey replaced corn syrup on the basis of percent solids in the corn syrup (honey at 33.6%; 1176 g). Honey was diluted to deliver the same amount of solids as the corn syrup in the formulation. Sugar analogue, a mix of sugars representative of those in honey, was incorporated on the basis of the average weight percentage of sugars in honey (i.e., fructose added at 45% and glucose added at 35%).

Table 1. EDTA and Corn Syrup French Salad Dressing Formulation Using Xanthan Gum Alone

French dressing using all xanthan	%	3500 g
HFCS	38.90	1361.50
soybean Oil	35.00	1225.00
120 grain vinegar	10.00	350.00
water	7.81	273.35
tomato paste	5.08	177.80
salt	2.50	87.50
polysorbate 60	0.20	7.00
garlic powder	0.10	3.50
oleoresin paprika	0.10	3.50
sorbic acid	0.10	3.50
xanthan gum	0.20	7.00
calcium disodium EDTA	0.01	0.35
total	100.00	3500.00

Two-ounce clear glass jars were filled with 50 g of dressing and flushed with nitrogen prior to sealing with polyvinyl lined phenolic caps. Jars were stored at 37 °C for 6 weeks and both 23 and 4 °C for up to 1 year.

Salad dressing particle size was determined using a Beckman Coulter Laser Diffraction Particle Size Analyzer (LS 13 320 Universal Liquid Module, Beckman Coulter, Inc., Fullerton, CA). Dressings were diluted with water (1:10) and measured in duplicate at 25 °C. Limits of the particle size measurement were 0.04 to 2000 μm.

Apparent viscosity was determined using a Brookfield Viscometer (Model RVT, Brookfield Engineering Laboratories, Stoughton, MA) at 25 °C, using spindle No. 4 and 150 g of dressing. Apparent viscosity was calculated by multiplying the deflection reading by the conversion factor supplied by the manufacturer.

Salad dressing (15 g) was measured into 20 mL vials and 1.5 mL microfuge tubes (1.5 g) in duplicate. Microfuge tubes were sonicated (Quantrex 210, L&R Ultrasonics, Kearny, NJ) for 3 min and stored at −20 °C for PV analysis the next day. Larger vials were sonicated for 1 min and centrifuged for 10 min at 11,000 rpm (14,500 × *g*) (RC2-B Sorvall, Newtown, CT). Vials were sonicated an additional 1 min and stored at −20 °C for pAV analysis the next day. Samples were thawed to room temperature prior to testing. Microfuge tubes were centrifuged for 10 min at 14,000 rpm (15,800 × *g*) (Eppendorf Centrifuge 5415C, Hamburg, Germany). Larger vials were centrifuged for 10 min at 11,000 rpm (14,500 × *g*).

Peroxide value was measured as described by Hornero-Méndez and others (*27*), on the basis of the standard method of the International Dairy Federation (*28, 29*). Fe(II) reacts with hydroperoxides in the sample to form Fe(III), which complexes with ammonium thiocyanate to form a reddish color, measured spectrophotometrically at 470 and 670 nm.

Aldehydes were measured using the para-anisidine value (pAV), determined by the AOCS Official Method Cd 18−90 (*30*). This pAV is often used to determine secondary products of oxidation and is frequently combined with other indicators to determine the extent of oxidation in oils.

Statistical analysis was performed using Statistical Analysis Systems (SAS) software (SAS Institute, Cary, NC, version 8.02) to determine analysis of variance (ANOVA) and Fisher's least significant difference (LSD) for each week of testing. Statistical significance was set at $p < 0.05$. SAS was also used for viscosity (ANOVA) and particle size (repeated measure ANOVA) data. Phenolic profiles were compared using Student's *t*-tests.

Table 2. Oxygen Radical Absorbance Capacity (ORAC) Values of Various Honeys[a]

floral source	ORAC (μmol TE/g)
NY buckwheat[b]	9.75 ± 0.48
Hawaiian Christmas berry[b]	8.87 ± 0.33
soy[b] (1996)	8.34 ± 0.51
blueberry	6.89 ± 0.20
avocado	6.51 ± 0.79
tupelo[b]	6.48 ± 0.37
blackberry (dark)	6.43 ± 0.37
saw palmetto	6.07 ± 1.27
gallberry	5.38 ± 0.04
clover	4.41 ± 0.78
cabbage	3.95 ± 0.42
sourwood	3.80 ± 0.47
sage	3.63 ± 0.47
eucalyptus	3.56 ± 0.26
fireweed[b]	3.09 ± 0.27
acacia[b]	3.00 ± 0.16
blackberry (light)	2.70 ± 0.48
orange blossom	2.36 ± 0.24
starthistle	1.75 ± 0.04

[a] Data expressed as mean μmol TE/g honey ± SEM ($n \geq 2$). [b] Data from Gheldof and others (*19*).

RESULTS

There was a wide range of antioxidant capacity in the 19 honeys evaluated, with ORAC values ranging from 1.75 to 9.75 μmol TE/g (**Table 2**). These values are consistent with previous literature reports of the antioxidant capacity of honey (*19*). ORAC value was one of the critical determinations used in the eventual selection of 2 of the 19 honeys, blueberry and clover, for formulation of the dressings to be evaluated. Blueberry and clover honeys have moderate ORAC values of 6.89 and 4.41 μmol TE/g honey, respectively.

Another key determinant of honey selection was the phenolic acid and flavonoid profiles generated using HPLC. Phenolic profiles of blueberry honey are shifted toward the phenolic acid end of the spectrum, while clover honey has a phenolic profile with peaks distributed across both the phenolic acid and flavonoid regions of the spectrum (**Figure 1**; **Table 3**). Because of a lack of literature reports on the metal chelation effects of honey, it is not known what quantity of phenolics would be required to effectively deter oxidation of these emulsions. Quantitation of known phenolics yielded ~0.45−0.6 mg identified phenolics/100 g salad dressing (honey formulations) as opposed to 6 mg EDTA/100 g salad dressing (in HFCS formulation).

Honey diastase activity was measured using Gothe's scale, with higher values indicating more diastase activity. Similar diastase activity was observed for blueberry and clover honey samples, 9.43 ± 0.12 and 9.72 ± 0.59, respectively. Reported honey diastase values range from 2.1 to 61.2, with an average of 20.8 (*31*).

Salad dressing formulations were initially made using a combination of xanthan gum and starch as thickening agents; however, emulsion instability was evident within the first week of storage. A new formulation using xanthan gum as a replacement for starch was created to stabilize the honey dressing emulsions. Salad dressings made with EDTA and sugar analogue were also reformulated using only xanthan gum as the thickening agent (see **Table 1** with formulation).

Median particle sizes ranged from 4.551 to 6.676 μm (**Table 4**). Mean particle sizes ranged from 7.064 to 124.0 μm (**Table 4**). Particle size distributions were mostly bimodal.

Apparent viscosity measurements were conducted on the day of formulation and after 12 weeks of storage at room temperature (**Table 5**). Apparent viscosities for sugar analogue and EDTA formulations were 4283 and 4367 centipoise, respectively (day 1), and decreased to 3233 and 3200 centipoise, respectively (week 12). Formulations incorporating blueberry and clover honeys were 4267 and 4150 centipoise, respectively (day 1), and 2650 and 2975 centipoise, respectively (week 12).

After 6 weeks of accelerated storage (37 °C), dressings made from the sugar analogue had significantly higher PVs than all other samples (**Table 6**). Dressings prepared using blueberry and clover honeys oxidized significantly less than sugar analogue samples, but more than the dressing made with EDTA. The differences between honey and EDTA formulations were not apparent at week 5. Under controlled room temperature (23 °C) conditions, the formulation containing the sugar analogue oxidized significantly more than other samples, followed by clover honey samples, then blueberry honey, and EDTA samples. These trends became significant at 24 weeks. Dressings held at 4 °C demonstrated trends similar to those held at 23 °C.

Accelerated storage (6 weeks) resulted in the highest aldehyde concentrations in sugar analogue formulations, followed by blueberry honey, clover, and EDTA formulations (**Table 7**). Room temperature storage resulted in the highest pAV for sugar analogue formulations, followed by blueberry honey and clover samples. Samples prepared with EDTA had lower values than all of the other samples. Under conditions of storage at 4 °C, pAV did not increase to nearly the same extent as they did at higher temperature storage. Sugar analogue formulations maintained significantly higher values than all other formulations, consistent with other storage conditions. The formulations containing honey and EDTA were similar until the last testing period, at which point clover honey samples had higher values than blueberry honey and EDTA formulations.

DISCUSSION

Selection of blueberry and clover honeys to use in salad dressing formulations was based upon a number of factors, including a strong emphasis on the phenolic profile and ORAC value (as measure of antioxidant capacity) of each honey. Blueberry honey had more phenolic acids than flavonoids, while the clover honey profile demonstrated a more even distribution between phenolic acids and flavonoids (**Figure 1**). It was important to our hypothesis to select/compare honeys with different phenolic profiles because of the fact that oils oxidize mechanistically differently than emulsions. Oil-based emulsions are prone to copper-catalyzed oxidation (*4*); thus, honeys with the potential to chelate metals would be ideal. Flavonoids act as free radical scavengers, peroxy radical scavengers, and as metal chelators (*14*). Phenolic acids act as chain-breaking inhibitors of oxidation and scavenge for peroxyl and hydroxyl radicals. Studies have indicated that flavonoids may be better metal chelators than phenolic acids (*32, 33*), suggesting that blueberry honey may have less metal chelating capabilities than clover honey. Honeys with phenolic profiles shifted toward the phenolic acid end of the spectrum may not protect an emulsion system to the same degree as honeys with a more even distribution of phenolic acids and flavonoids.

Selecting honeys based only on the phenolic profile is not practical, however. Even though research indicates that phenolic compounds are effective antioxidants, the overall antioxidant activity of honey is attributed to several components, including phenolics, peptides, organic acids, and Maillard browning reaction products (*19*). One cannot assign the antioxidant properties of honey in food systems to a specific single phenolic component. Antioxidant capacity testing helps predict how

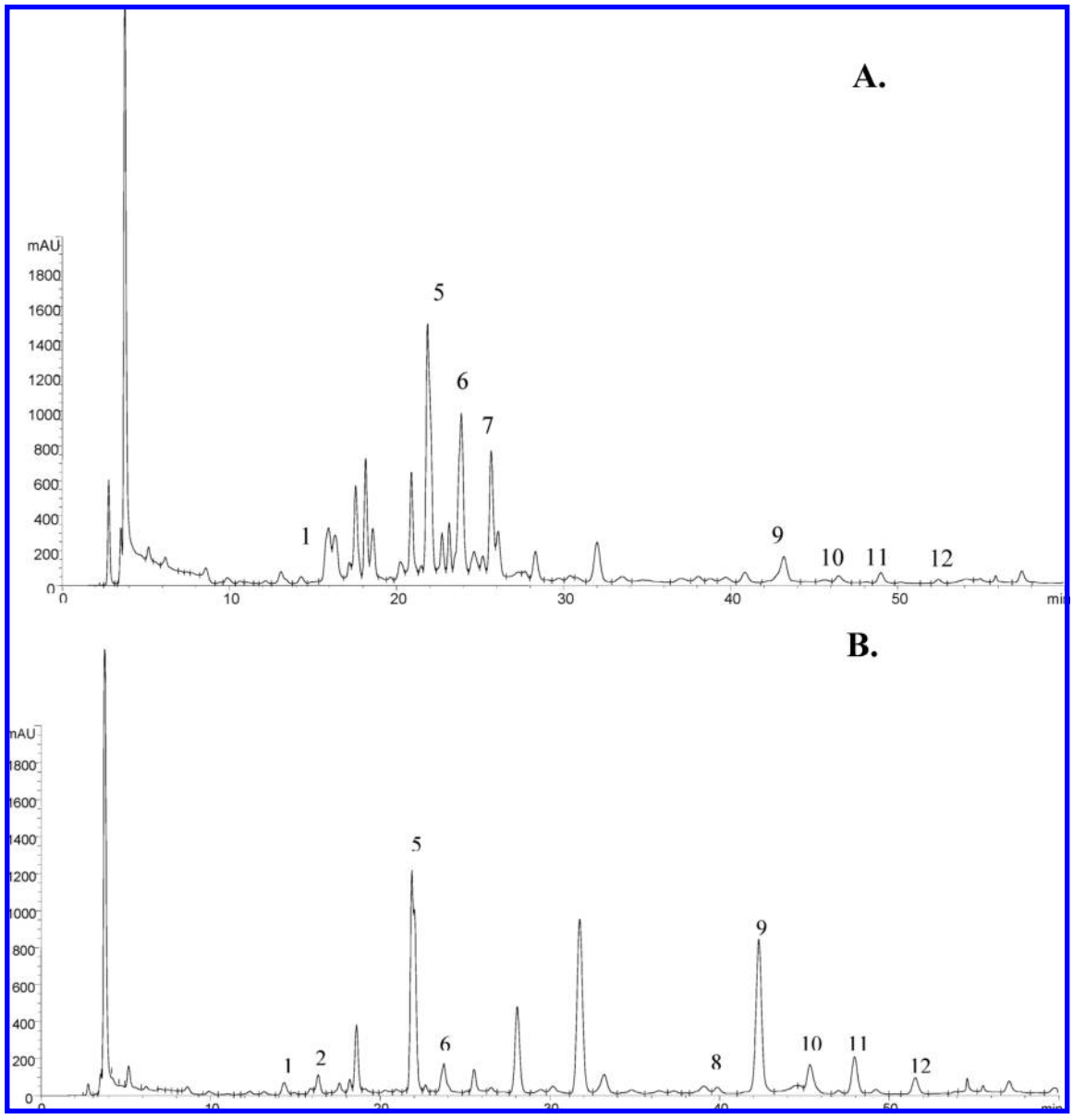

Figure 1. HPLC chromatograms of blueberry (**A**) and clover (**B**) honey extracts at 285 nm. For peak identification, see **Table 2**.

Table 3. Phenolic Acid and Flavonoid Content of Clover and Blueberry Honeys[a]

peak	MW (mol/g)	phenolic	blueberry	clover
1	138	*p*-hydroxybenzoic acid	2.319 ± 0.464 a	1.816 ± 0.096 a
2	168	vanillic acid	1.307 ± 0.156 a	0.996 ± 0.028 b
5	164	*p*-coumaric acid	3.194 ± 0.046 a	2.038 ± 0.075 b
6	264	*cis,trans*-abscisic acid	4.990 ± 0.184 a	3.222 ± 0.171 b
7	148	cinnamic acid	0.944 ± 0.082 a	0.386 ± 0.042 b
8	302	quercetin	1.223 ± 0.496 a	1.171 ± 0.286 a
9	256	pinocembrin	1.491 ± 0.273 a	1.717 ± 0.082 a
10	286	kaempferol	0.552 ± 0.197 b	1.090 ± 0.116 a
11	254	chrysin	0.676 ± 0.153 a	0.665 ± 0.075 a
12	270	galangin	1.092 ± 0.626 a	0.556 ± 0.096 a
		total identified phenolic content	17.788	13.657

[a] Data expressed as mg/kg honey ± standard deviation. Means sharing the same letter in the same row are not significantly different ($p < 0.05$).

effective honey varieties will be as free radical scavengers. ORAC testing indicates the level of antioxidant protection provided and reflects the capacity of various antioxidant components in honey. However, the ORAC assay serves as an indicator of the ability of the antioxidants in the test sample to quench peroxyl free radicals; one must keep in mind that the radicals generated in food systems, such as French salad dressing, may be more complex. Blueberry and clover honeys had ORAC values in the range of honeys previously demonstrated protective against oxidation in foods. For example, previous research indicated that soy honey with an ORAC value of 5.9 μmol TE/g was effective at preventing lipid oxidation in cooked ground turkey (*9*).

Table 4. Particle Size of Salad Dressings[a]

sample	mean (μm)[b]	median (μm)[b]
EDTA[c]	7.064 ± 1.637 a	5.094 ± 0.255 a
EDTA	124.0 ± 7.977 d	6.676 ± 1.792 a
sugar analogue[c]	32.18 ± 41.96 a,b	6.474 ± 2.632 a
sugar analogue	100.4 ± 18.27 c,d	4.551 ± 0.097 a
blueberry	71.68 ± 22.28 b,c	5.394 ± 0.261 a
clover	87.45 ± 30.08 c,d	5.338 ± 0.574 a

[a] Average of 3 batches measured in duplicate. Means sharing the same letter in the same column are not significantly different ($p < 0.05$). [b] Value ± standard deviation. [c] Formulas using starch and xanthan gum.

Table 5. Apparent Viscosity of Salad Dressings[a]

sample	centipoise (mPa·s) day 1	centipoise (mPa·s) week 12
EDTA[b]	2333 ± 252 a A	2990 ± 193 abc B
EDTA	4367 ± 539 bcd A	3200 ± 283 bc B
Sugar Analogue[b]	2233 ± 29 a A	2650 ± 180 ac B
Sugar Analogue	4283 ± 236 cd A	3233 ± 58 bc B
Blueberry	4267 ± 115 bcd A	2650 ± 71 ac B
Clover	4150 ± 150 bc A	2975 ± 35 abc B

[a] Values reported at 20 rpm. Value ± standard deviation. Means sharing the same upper case letter in the same row are not significantly different. Those sharing the same lower case letter in the same column are not significantly different ($p < 0.05$). [b] Formulas using starch and xanthan gum.

Diastase (amylase) hydrolyzes starch into short glucose chains and glucose monomers (*34*). This could be problematic in a salad dressing system due to the resultant decrease in viscosity of a starch-thickened product. Ultimately, this would lead to rapid destruction of an emulsion. Starches and gums are used in dressings to stabilize against creaming (*35*), on the basis of the increase in viscosity of the continuous phase. Honey diastase activity could potentially hydrolyze the starch and subsequently break the emulsion. Honey diastase values were low compared to those reported in the literature (2.1−61.2, with an average of 20.8) (*30*), perhaps due to heat treatment during processing or floral variety. Initially, the low diastase activity of the honeys was not a major concern in the formulation.

Another issue of consideration in formulation and selection of honeys was economic factors. Blueberry is not a well-known honey floral variety; however, blueberries have been well studied as a natural source of antioxidants. The antioxidant capacity of blueberries indicates that they are a promising source of antioxidants in the diet (*36*−*38*), with higher antioxidant capacities than other small fruits, such as strawberries and raspberries, although the antioxidant components of blueberry fruit and honey would be expected to be different. Positive research and popular press articles on blueberries as a dietary source of antioxidants could potentially increase consumer acceptance of a product containing blueberry honey. Clover honey is well known by honey consumers and is also widely available. Blueberry and clover honeys are mild in taste and medium in color, which would minimize their impact on flavor and appearance of the salad dressing. These factors were strongly considered in the selection of clover and blueberry honeys to incorporate into the dressing formulations.

Salad dressing is often formulated using starch as a thickener and stabilizer; in this study, dressings made with starch and honey were not physically stable. Dressings formulated with blueberry and clover honeys experienced broken emulsions within the first week of storage at room temperature. Although diastase activity of the selected honeys was low compared to average diastase activity, diastase was speculated to be responsible for emulsion instability due to starch thinning since the dressings formulated with sugar analogue and EDTA remained stable. Studies have shown that a commercially available honey (blend from China) with a diastase value of 10.6 was able to cause starch thinning and viscosity loss in a starch mixture (*39*). Challenges encountered in using starch were overcome by reformulating the dressings using xanthan gum as the thickener and stabilizer. Sugar analogue and EDTA dressings were also reformulated using only xanthan gum.

Despite reformulation without starch, honey salad dressing samples exhibited weaker emulsions, and separation of oil from the dressings for analytical testing was less difficult in comparison to dressings containing EDTA and sugar analogue. After the first few weeks of storage at room temperature, dressing samples containing honey did not require sonication or centrifugation before freezing; oil was easily separated after thawing and centrifuging. EDTA and sugar analogue samples continually needed to be sonicated and centrifuged prior to freezing. Further investigation into the weaker emulsions of honey salad dressing formulations versus those created with EDTA and sugar analogue is recommended.

Lipid droplet size within the dressing (particle size) was determined at the time of formulation and differences in mean particle size were apparent between the formulations incorporating starch/xanthan gum or xanthan gum alone (**Table 4**). Samples incorporating starch had smaller mean particle sizes as compared to those incorporating xanthan gum alone. However, median particle sizes were similar for all formulations. This may be attributed to dispersion of the droplets. Most samples that incorporated starch had monodispersed oil droplets, with one distribution around the mean. Samples incorporating xanthan gum showed distributions that were polydispersed, with at least two distributions of oil droplet particle sizes. Polydispersion resulted in higher mean particle size; however, median particle size was consistent over all samples. The largest distribution was around the median. Polydispersion of oil droplets was noted in literature for systems incorporating gums (*40, 41*). Increasing the concentration of xanthan gum in an emulsion was shown to increase viscosity, possibly reduce the efficiency of homogenization, and cause the formation of larger oil droplets (*41*).

Average apparent viscosities of starch-containing samples were approximately 50% of the apparent viscosities of the xanthan gum formulations at day one (**Table 5**). At week 12, samples were less viscous; those containing honey showed more dramatic reductions in viscosity than those containing EDTA or sugar analogue. This loss in viscosity suggests that although the xanthan gum formulation was more stable than the formulation containing starch, the honey had an adverse impact on stability. Interactions of honey components with xanthan gum and resultant changes in emulsion stability need to be further studied to understand this phenomenon.

Peroxide values of salad dressings reveal that EDTA and blueberry honey were the most effective antioxidants in these formulations, followed by clover honey (**Table 6**). Dressing formulation was originally optimized for the use of EDTA; EDTA samples were thus expected to resist oxidation over time. Sugar analogue samples contained no added antioxidants to help prevent oxidation and were expected to oxidize faster than samples including either EDTA or honey. Blueberry and clover honeys provided more protection against oxidation than the sugar analogue; blueberry honey provided slightly more protection than clover honey, with significant differences seen throughout storage at 23 and 4 °C. Blueberry honey sample measurements had smaller standard deviations than the clover honey samples. At 37 °C, the PV of clover honey samples increased quickly within the first three weeks and then decreased at week five with a final reading slightly higher than the blueberry honey samples. At room temperature, clover samples showed higher standard deviations compared to the blueberry samples, indicating that the batches were not consistent.

One of the goals of oil refining is to ensure that the oil has a PV less than 1 meq/kg (*42, 5*); however, a limit is not set for a product throughout its shelf life. There are limitations to the use of PV as a means of determining the shelf life of oils (*42*); yet it is a commonly used indicator of the extent of oil oxidation. Deterioration of flavor in soybean oil may occur at PV of 5−10

Table 6. Peroxide Value of Samples Held at 37, 23, and 4 °C[a]

37 °C	EDTA	SA	Clover	Blueberry
week 0	1.026 ± 1.067 b	2.515 ± 0.060 a	0.575 ± 0.161 b	0.452 ± 0.025 b
week 1	1.018 ± 0.565 b	4.406 ± 3.592 a	1.464 ± 1.755 b	0.480 ± 0.254 b
week 3	2.059 ± 0.443 b	14.286 ± 11.297 a	10.428 ± 7.158 a	2.835 ± 1.744 b
week 5	2.548 ± 0.659 b	20.546 ± 9.502 a	7.940 ± 6.504 b	7.299 ± 3.667 b
week 6	2.969 ± 0.657 c	28.895 ± 6.195 a	9.227 ± 4.098 b	7.729 ± 2.305 b

23 °C	EDTA	SA	Clover	Blueberry
week 0	1.026 ± 1.067 b	2.515 ± 0.060 a	0.575 ± 0.161 b	0.452 ± 0.025 b
week 6	1.113 ± 0.620 b	17.884 ± 10.944 a	3.986 ± 4.577 b	1.100 ± 0.331 b
week 12	2.365 ± 0.33 b	22.688 ± 9.291 a	6.117 ± 7.251 b	3.359 ± 2.075 b
week 24	8.036 ± 1.19 b	34.995 ± 15.8 a	37.235 ± 7.49 a	7.728 ± 2.443 b
week 41	14.689 ± 2.639 c	63.694 ± 27.498 a	41.847 ± 11.666 b	5.13 ± 6.619 c
week 52	54.711 ± 6.756 c	334.183 ± 124.359 a	161.138 ± 112.568 b	40.862 ± 14.397 c

4 °C	EDTA	SA	Clover	Blueberry
week 0	1.026 ± 1.067 b	2.515 ± 0.060 a	0.575 ± 0.161 b	0.452 ± 0.025 b
week 6	0.685 ± 0.533 b	4.426 ± 1.107 a	1.001 ± 0.277 b	0.457 ± 0.050 b
week 12	0.864 ± 0.589 c	8.227 ± 1.214 a	2.611 ± 0.840 b	0.706 ± 0.098 c
week 24	1.383 ± 0.691 c	24.355 ± 3.778 a	9.065 ± 0.918 b	1.553 ± 0.20 c
week 41	2.006 ± 0.527 c	43.877 ± 10.927 a	21.142 ± 2.46 b	3.181 ± 0.391 c
week 52	−4.998 ± 8.041 b	102.781 ± 55.395 a	30.134 ± 30.206 b	−0.525 ± 4.465 b

[a] Peroxide value expressed as meq peroxide/kg sample ± standard deviation. Means sharing the same letter within each row are not significantly different ($p < 0.05$).

Table 7. *p*-Anisidine Value for Samples Held at 37, 23, and 4 °C[a]

37 °C	EDTA	SA	Clover	Blueberry
week 0	0.664 ± 1.498 b	2.611 ± 0.111 a	2.534 ± 0.941 a	1.425 ± 0.443 b
week 1	1.592 ± 0.269 d	4.236 ± 0.454 a	2.788 ± 0.140 c	3.364 ± 0.472 b
week 3	2.413 ± 0.481 c	6.559 ± 1.125 a	5.036 ± 0.579 b	4.715 ± 0.824 b
week 5	2.727 ± 0.484 c	9.954 ± 3.168 a	7.528 ± 1.679 b	6.058 ± 0.791 b
week 6	2.979 ± 0.213 d	14.476 ± 1.163 a	8.965 ± 1.156 c	11.841 ± 3.652 b

23 °C	EDTA	SA	Clover	Blueberry
week 0	0.664 ± 1.498 b	2.611 ± 0.111 a	2.534 ± 0.941 a	1.425 ± 0.443 b
week 6	1.999 ± 0.104 d	4.107 ± 0.127 a	3.026 ± 0.154 b	2.603 ± 0.249 c
week 12	2.307 ± 0.382 d	5.95 ± 0.849 a	5.228 ± 0.416 b	3.811 ± 0.303 c
week 24	2.499 ± 1.12 b	7.511 ± 1.763 a	7.547 ± 0.817 a	7.44 ± 0.622 a
week 41	3.697 ± 0.153 c	16.987 ± 4.437 a	12.092 ± 2.54 b	11.217 ± 4.22 b
week 52	3.563 ± 0.399 c	23.387 ± 4.293 a	18.056 ± 1.63 b	21.613 ± 3.36 ab

4 °C	EDTA	SA	Clover	Blueberry
week 0	0.664 ± 1.498 b	2.611 ± 0.111 a	2.534 ± 0.941 a	1.425 ± 0.443 b
week 6	1.620 ± 0.200 c	2.582 ± 0.684 a	2.021 ± 0.149 bc	2.207 ± 0.335 ab
week 12	1.663 ± 0.483 c	3.021 ± 0.644 a	2.241 ± 0.093 b	2.127 ± 0.264 b
week 24	2.323 ± 0.435 bc	3.711 ± 0.381 a	2.869 ± 0.38 b	2.002 ± 0.826 c
week 41	2.326 ± 0.311 b	4.409 ± 0.272 a	2.475 ± 0.698 b	1.786 ± 1.04 b
week 52	2.185 ± 0.61 c	4.925 ± 0.426 a	4.17 ± 0.712 b	2.604 ± 0.346 c

[a] Means sharing the same letter within each row are not significantly different ($p < 0.05$).

meq/kg (*43*). Fried chips with a PV of 2.5 meq/kg may indicate oxidation and instability (*44*).

Trends in pAV reflected those of the PV analyses (**Table 7**). Samples containing EDTA and blueberry honey generally oxidized to a lesser extent than samples containing clover honey. Sugar analogue samples oxidized to a greater degree than the other samples. During storage at 4 °C, pAVs did not change dramatically over the course of the study. The pAV measures secondary products of oxidation, particularly conjugated dienals and 2-alkenals. Primary products of oxidation may not have broken down into secondary products at 4 °C. Schnepf and others (*42*) concluded that PV was more sensitive than pAV testing. This conclusion was supported by our findings.

In conclusion, the initial objective of this research was to select honeys for use in the protection of oil in a salad dressing system against oxidation, while providing sweetening potential. Honeys were selected for use in salad dressings based primarily on phenolic profiles and antioxidant capacity. French salad dressings incorporating clover or blueberry honey as the antioxidant source experienced inhibition of lipid oxidation (according to PV and pAV analyses) in comparison to salad dressings containing the sugar analogue with no added antioxidant. This research adds to a growing body of

evidence of honey's potential to protect against oxidation in various food systems, in this case, emulsion-based dressings, in addition to providing a natural source of sweetening potential. Using honey to create such formulations may help to replace synthetic antioxidants such as EDTA and thus enhance consumer acceptance.

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