Food Chemistry 123 (2010) 794–799

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of thiobarbituric acid reactive substances in microencapsulated products

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ARTICLE INFO

Article history:
Received 6 July 2009
Received in revised form 26 March 2010
Accepted 2 May 2010

Keywords:
Lipid oxidation
TBARS
Complete dissolution
Microencapsulation
Polyunsaturated fatty acids

ABSTRACT

Food samples are usually extracted or distillated before quantification of thiobarbituric acid reactive substances (TBARS) for evaluation of secondary lipid oxidation, which is a challenge for microencapsulation projects with limited sample quantities. Our approach was to use a ternary solvent mixture of 1-butanol, isopropanol, and 0.5 M HCl (in water) at a proportion of 2:2:1 (v/v/v) to completely dissolve microcapsules of fish oil. The solvent system allowed the construction of highly linear standard curves with good sensitivity: 0.4–13 μM 1,1,3,3,-tetramethoxypropane as a TBA reactive standard with a R^2 value of 99.86%. For a sample of fish oil coated by corn zein, only 40 mg of the sample was needed to obtain reliable and repeatable TBARS values. Further, several common encapsulation materials gave low interferences to the assay. The proposed approach, with the above qualities along with good precision and accuracy, may be applicable to a variety of relevant bioactive compounds and encapsulation materials.

1. Introduction

Polyunsaturated fatty acids (PUFAs) have shown health-benefiting attributes such as reduced risks of coronary heart diseases, hypertension, thrombosis, inflammations, and rheumatoid arthritis (Stone, 1997). As such, much work has been focused on incorporating PUFAs in various food products. The chemical structure of PUFAs determines that they are highly susceptible to oxidation during processing, transportation, and storage. Peroxides are primary products of lipid oxidation and are further decomposed to secondary products such as aldehydes, ketones, alcohols, acids, and hydrocarbons (Angelo, 1996). These so-called secondary oxidation products can change food quality attributes of colour, texture, flavour, and odour (Fernández, Pérez-álvarez, & Fernández-López, 1997).

Microencapsulation is a well-recognised approach to protect PUFAs against oxidation caused by environment factors such as oxygen, light, and humidity, as well as chemical factors such as cupric ions that are catalysts of oxidation reactions (Thautwein, 2001). Many processes have been developed to incorporate the oil body in particulate structures. Conventionally, oil can be prepared into emulsions that can be used in products such as beverages directly or prepared into the powdered form by further coating oil droplets with other materials. Preparation of powdered products can be achieved by processes such as supercritical anti-solvent, spray-freeze drying, spray drying, fluidized bed drying, and freeze-drying. The powdered products can be incorporated in various products, dry or wet.

Many materials have been used to coat oil droplets using spray drying: dairy proteins, gelatin, maltodextrins, starch, gum acacia, alginate, among others (Heinzelmann, Franke, Jensen, & Haahr, 2000; Hogan, O'Riordan, & O'Sullivan, 2003). In preliminary stages of microencapsulation projects, the mass of materials may be limited during researching formulations and process parameters, e.g., only several grams of powdered products. The evaluation of a product, on the other hand, may require several treatments to test oxidative stability of encapsulated PUFAs during storage for different durations at certain environmental conditions such as temperature, relative humidity, and solvent chemistry. Assay methods are thus needed to reliably and accurately test samples with small quantities.

Quantification of secondary oxidation products has been described in many methods to target various chemicals. Testing thiobarbituric acid reactive substances (TBARS) is one of the conventional methods. The assay is based on the reaction of 2-thiobarbituric acid (TBA) and malonaldehyde or malonaldehyde-type products. The reaction results in a coloured compound that corresponds to a maximum absorbance at a wavelength of ca. 530 nm (Pike, 2003). However, compounds other than malonaldehyde-type products may also react with TBA. The specificity of assays is thus usually poor, and the results are expressed as TBARS.

The first spectrophotometric determination of TBARS was described by Kohn and Liversedge (1944) in a paper for evaluation

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doi:10.1016/j.foodchem.2010.05.012

of oxidation of meat products. Since then, many protocols and modifications have been developed to improve the determination of TBARS. For edible oil samples or lipid extracts, the TBA test is simplified in that samples can be directly dissolved in butanol before reaction with TBA. Testing of TBARS for complex foodstuffs may be challenging because malonaldehyde can be bound to various constituents of food matrices. Three general approaches have been employed to extract malonaldehyde from precursors or bound forms from food samples before extraction for reaction with TBA for spectrophotometry tests: (1) aqueous acid extraction (Salih, Smith, Price, & Dawson, 1987; Tarladgis, Pearson, & Dugan, 1964; Witte, Krause, & Bailey, 1970), (2) distillation at boiling conditions after addition of distilled water (Ke, Cervantes, & Robles-Martinez, 1984; Yamauchi, Nagai, & Ohashi, 1982), and (3) extraction of lipid portion (Pikul, Leszczynski, & Kummerow, 1983; Younathan & Watts, 1960). The TBA test result is thus dependent on the extraction and fractionation conditions applied. Alternatively, a food sample can be heated with a TBA solution and the pigment formed can be extracted with butanol or a butanol/pyridine solution for spectrophotometry (Pokorny & Dieffenbacher, 1989; Sinnhuber, Yu, & Yu, 1958; Turner et al., 1954). Heating during sample preparations however may result in additional lipid oxidation that is not indicative of sample properties. Therefore, although many protocols are available in literature, the optimal conditions for isolation of malonaldehyde-relevant products may still be needed for specific food samples. This aspect adds another challenge to lab-scale microencapsulation projects with limited sample quantities.

The objective of this work was to explore the possibility of complete dissolution of microencapsulated products for reliable and reproducible quantification of TBARS using spectrophotometry. We found that a ternary solvent mixture composed of n-butanol, isopropanol, and 0.5 M HCl was capable of dissolving fish oil as well as an exemplary spray-dried fish oil product coated by corn zein (prolamines). The assay procedures required only small quantities (as little as 40 mg) for reliable TBA tests. Because cyclodextrins, chitosan, polyethylene glycol, methylcellulose, and hydroxypropylmethylcellulose are other examples of common encapsulation carrier materials, we continued to test if the ternary solvent mixture was able to dissolve these materials, as well as the subsequent interference on the TBA test.

2. Materials and methods

2.1. Materials

Ethanol, isopropanol, malonaldehyde bis(dimethyl acetal) – also named as 1,1,3,3-tetramethoxypropane (TMP) or malonaldehyde tetramethyl acetal, 2-thiobarbituric acid (TBA), 2,6-di-tert-butyl-4-methylphenol (BHT), soybean lecithin (catalog number AC41310), and polyoxyethylene (20) sorbitan monopalmitate (Tween® 40), and purified zein were purchased from Acros Organics (Morris Plains, NJ). 1-butanol, trichloroacetic acid, chitosan (medium molecular weight) and poly(ethylene glycol) (PEG P3640) were obtained from Sigma–Aldrich (St. Louis, MO). Corn starch (Novelose 240) and corn syrup solids (N-Tack) were provided by National Starch and Chemical Company (Bridgewater, NJ). Methylcellulose (product TIC Pretested® TICACEL MC LV) was provided by TIC Gums Company (White Marsh, MD). Hydroxypropyl methylcellulose (product METHOCEL E50) was a kind gift from the Dow Chemical Company (Midland, MI). Fish oil, labelled as "Omega 3 TG food grade fish oil," was generously supplied by Ocean Nutrition Canada (Dartmouth, Nova Scotia, Canada). Fish oil was stored in a –20 °C freezer and thawed right before use; after each use, the head space of container was flushed by nitrogen before being returned to the –20 °C freezer. Distilled water (catalog number S80239) was a product from Fisher Science Education (Hanover, IL). Whey protein isolate was a product from Hilmar Cheese Company (Hilmar, CA). Soy protein isolate (SPI) was extracted from defatted soy flour using a literature protocol (Wang, Ma, Pagadala, Sherrard, & Krishnan, 1998).

2.2. Encapsulation of fish oil

A fish oil emulsion was prepared by emulsifying a mixture with 4% w/v fish oil and 0.2% w/v lecithin in 250 mL 90% (v/v) aqueous ethanol at 1×10^4 rpm for 5 min using a high speed homogenizer (Cyclone I.Q.^{2}, The VirTis Company, Inc., Gardiner, NY). The homogenizer was equipped with a 20 mm diameter rotor/stator shaft assembly that had a flow-through head with slotted orifices of a width of 1 mm and a height of 10 mm. After emulsification, corn zein was dissolved in the emulsion to a concentration of 16% w/v, and the emulsion was then spray-dried with a B-290 Mini Spray Dryer (BÜCHI Labortechnik AG, Postfach, Switzerland). The inlet and outlet temperatures were set at 110 and 65–70 °C, respectively, and the feed rate was 8.00 mL/min. The spray-dried powders were collected and stored in a −20 °C freezer until analysis.

2.3. Solvents and stock solutions

2.3.1. Ternary solvent mixture

The solvent mixture was prepared by mixing 100 mL 1-butanol, 100 mL isopropanol and 50 mL 0.5 M HCl (in water) until a monophasic appearance. The solvent mixture was stored in an amber glass bottle before following analyses.

2.3.2. TBA stock solution

The TBA stock solution was composed of 15 g trichloroacetic acid, 0.75 g TBA, and without or with an appropriate amount of BHT dissolved in 100 mL of the above ternary solvent mixture. The stock solution was stable more than 1 week when stored at 4 °C.

2.4. Procedures of TBA tests

The procedures of TBA tests followed those of the literature (Ronald, 2001), with some modification. A powdered material, i.e., TMP (as a standard), the spray-dried fish oil sample or a conventional microencapsulation material, was directly weighed into a 15 mL screw-cap centrifuge tube (Corning Inc., Lowell, MA), followed by addition of the above TBA stock solution to a total volume of 10 mL. The sample was vortexed until a clear solution. The centrifuge tubes were incubated for 2 h in a 95 °C water bath, followed by immediately cooling to room temperature in a water bath. The cooled samples were measured for absorbance in the visible light spectrum (380–800 nm) using a UV/Vis spectrophotometer (model BioMate 5, Thermo Fisher Scientific, Pittsburgh, PA). Glass cuvettes with a light path length of 1 cm were used in all measurements, and the ternary solvent mixture was used as a reagent blank. The absorbance data were the averages of at least triplicate determinations.

2.5. Standard curve and calculation of TBARS

The TMP was used as a standard in the TBA test (Ronald, 2001), as noted above. One mole of TMP produces an equal mole of chromogen according to stoichiometry. The TMP was prepared at a concentration range of 0.2–20.0 μM using the procedures in the above TBA test. The absorbance values at 532 nm (A_{532}) were used to construct standard curves for correlation to TMP concentrations based on Beer's law:

$$A_{532} = \varepsilon\, b\, C \quad (1)$$

where A_{532} is the absolute absorbance at 532 nm after calibration with an appropriate blank, ε is the molar absorptivity or molar extinction coefficient ($M^{-1}\,cm^{-1}$), b is the length of light path (1 cm), and C is the molar concentration of chromogen (M) that is quantitatively identical to the mole concentration reduction of reacted TMP based on stoichiometry.

The TBARS value of a sample can then be calculated based on the following equation:

$$\text{TBARS} = \frac{A_{532}/(\varepsilon b)}{M \times \text{wt.}\%} \times 10^7 \quad (2)$$

where the numerator is the equivalent TMP concentration (in M) determined from Eq. (1), M is the sample mass used in an assay (in mg), wt.% is the weight percentage of oil in the encapsulated sample. The conversion factor 10^7 has taken into account the volume of assay solution (10 mL) within which the encapsulated sample is dissolved. The TBARS as defined has a unit of mmol malonaldehyde equivalent/kg oil, simplified as mmol/kg oil hereafter.

2.6. Statistical analysis

The linear regression of standard curves was completed with Origin software (version 6.0, OriginLab, Northampton, MA). The analysis of variance (ANOVA) for linear regression was performed using the F-test, with $p < 0.05$ indicating the differences were statistically significant.

3. Results and discussion

3.1. Solvent selection

Our goal was to find a solvent system that is able to dissolve capsules and suitable for TBA tests. For capsules, the solvent system should dissolve both the carrier material and the encapsulated compound (fish oil). Carrier materials include those forming the structures, e.g., polymers and those that facilitate structure formation or modulate interactions between the carrier compounds and encapsulated compounds, e.g., surfactants. A large variety of carrier materials have been used to encapsulate fish oil and these materials vary significantly in physicochemical properties. For TBA tests, a desirable solvent system should be able to dissolve all TBA test reagents, i.e., TBA, TMP, trichloroacetic acid, HCl, and BHT. In addition, because heating is required for the reaction to form chromogen, the solvent system should also be able to maintain stability during heating, for example, a constant volume and the maintained solubility of all compounds during the reaction.

Both fish oil and zein are not soluble in water. Based on our experience, the zein sample used is soluble in ~60–90% aqueous alcohol (ethanol, isopropanol or butanol) and 60–100% aqueous methanol, and fish oil is soluble to some extent (~2%) in 90% isopropanol but very little in aqueous or 100% ethanol or methanol (Zhong, Tian, & Zivanovic, 2009). Dimethyl sulfoxide and dimethylformamide may be able to dissolve some biopolymers but the high polarity of these two solvents is a challenge to dissolve non-polar fish oil. Therefore, it is difficult to find a single solvent that is capable of dissolving both zein and fish oil; binary solvent mixtures such as 90% isopropanol and 90% butanol are possible options for our fish oil capsules but may be problematic to some more polar carrier materials such as water-soluble biopolymers.

Conversely, much work has been done in the pharmaceutical field because of the need to dissolve drugs of varying polarity. A lot of attention has been paid to ternary solvent mixtures that are able to dissolve many compounds that would otherwise be impossible for single solvents or binary solvent mixtures (JoubanGharamaleki, Clark, & Acree, 2000). In fact, a ternary mixture of chloroform, methanol, and an aqueous 0.88% KCl solution at proportions of 8:4:3 (v/v/v) was used to purify animal fat (Folch, Lees, & Stanley, 1957) and fish oil (Nordback, Lundberg, & Christie, 1998) from the corresponding resources. The mixture contained 20% water that should also be able to dissolve some hydrophilic biopolymers. However, the low boiling points of chloroform, 61.2 °C and methanol, 64.7 °C (NIST, 2009) may not be suitable for TBA tests requiring heating at 95 °C for 2 h. When ethanol was tried in our preliminary screenings, the volume of assay system was significantly reduced after TBA tests. A ternary mixture of 1-butanol, isopropanol, and 0.5 M HCl (in water) at a volume ratio of 2:2:1 (or 40%:40%:20%) was found to be able to dissolve appreciable amounts of corn zein, fish oil, and other conventional microencapsulation materials described below. The 0.5 M HCl, instead of deionised water, was used because the acid is used in conventional TBA tests (Ronald, 2001). The volume of the ternary solvent mixture maintained practically unchanged after TBA tests. Although not tested, the ratio of the three solvents may be changed to accommodate different materials, analysed similarly as below for TBA tests.

3.2. Absorption spectra of reaction products from TMP standard solutions

The TMP or its tetraethoxy analog is commonly used as a standard in the TBA tests (Bergamo, Fedele, Balestrieri, Abrescia, & Ferrara, 1998). Under acidic conditions, these acetals are hydrolysed to 1,3-propanedial (i.e., malonaldehyde) that subsequently reacts with TBA to produce a pinkish, fluorescent chromogen (conjugates of malonaldehyde and TBA) at an elevated temperature such as 95 °C (Bergamo et al., 1998). Fig. 1 represents the absorption spectra (from 400 to 800 nm) of the reaction products when TMP was used at a concentration of 2–16 μM in the TBA test. The absorption peaks centred on a wavelength of 532 nm, corresponding to the pink pigment as a result of reaction between malonaldehyde and TBA. The characteristic absorption wavelength was consistent with the literature (Pike, 2003). At the lowest TMP concentration tested (2 μM), the A_{532} was 0.29.

The A_{532} values were used to construct a standard curve and a linear regression was performed according to Eq. (1). The coeffi-

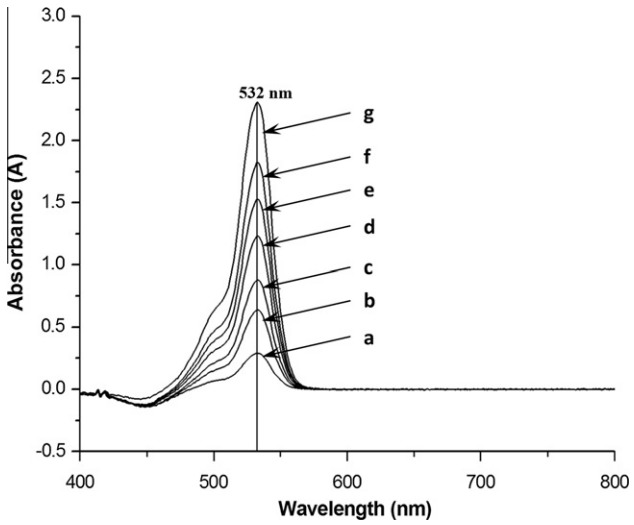

Fig. 1. Absorption spectra of the reaction products when different concentrations of 1,1,3,3-tetramethoxypropane (TMP) reacted with thiobarbituric acid (TBA). The curves represent systems with a TMP concentration of (a) 2, (b) 4, (c) 6, (d) 8, (e) 10, (f) 12 and (g) 16 μM, respectively.

cient of determination (R^2) was 0.998, and the slope was 0.153 μM^{-1} when the TMP concentration was used in units of μM, which corresponds to a molar absorptivity of 1.53×10^5 M^{-1} cm^{-1}. The molar absorptivity is ~6 times of a literature value of 2.58×10^4 M^{-1} cm^{-1} (Sheu, Chen, Tseng, Chen, & Tsai, 2003), indicating an improved sensitivity from our assay system.

3.3. Applicability to encapsulation products

The 40 mg of fish oil capsules was readily dissolved in the TBA stock solution. After following TBA test procedures, the absorption spectrum of the reaction product is presented in Fig. 2A, along with the absorption spectrum of a control sample prepared by using zein only, with an amount equivalent to expectation from formulation, i.e., 79.2% of capsules, assuming fish oil, lecithin and zein precipitated proportionally during spray drying. Both curves showed a significant absorbance at a wavelength shorter than ca. 550 nm and had a peak centred on 532 nm. The A_{532} for samples of fish oil capsules and zein alone was 0.185 and 0.087, respectively. After calibrating the absorption spectrum of the fish oil capsule sample by that of the zein sample, the absorption spectrum only had a peak centred on 532 nm; outside the peak, the absorbance was lower than 0.04 (Fig. 2A). Further, because phospholipids are known to interfere with the TBA assay using regular reagents and procedures (Kosugi, Kato, & Kikugawa, 1987; Witz, Lawrie, Zaccaria, Ferran, & Goldstein, 1986), the absorption spectrum was evaluated after the TBA test using the amount of lecithin equivalent to that expected in fish oil capsules. No appreciable absorbance (A_{532} = 0.075) was observed at a wavelength around 532 nm (Fig. 2B), indicating no need to calibrate the absorbance of fish oil capsule sample due to lecithin. An additional test using 2 mg of Tween 40 also showed negligible absorbance (A_{532} = 0.053) at a wavelength nearby 532 nm (Fig. 2B, bottom curve).

Eight other common encapsulation carrier materials were then examined: methylcellulose, hydroxypropylmethylcellulose, chitosan, corn starch, corn syrup, polyethylene glycol, SPI and whey protein isolate. The TBA stock solution was able to dissolve these compounds at a concentration of 4 mg/mL; a higher solubility may be possible but was not examined in this study. After TBA tests, the absorption spectra from these materials are presented in Fig. 3. Only SPI showed absorbance around 532 nm, possibly due to oxidised products present in the SPI powder. Nevertheless, calibration of products containing SPI can be performed similarly

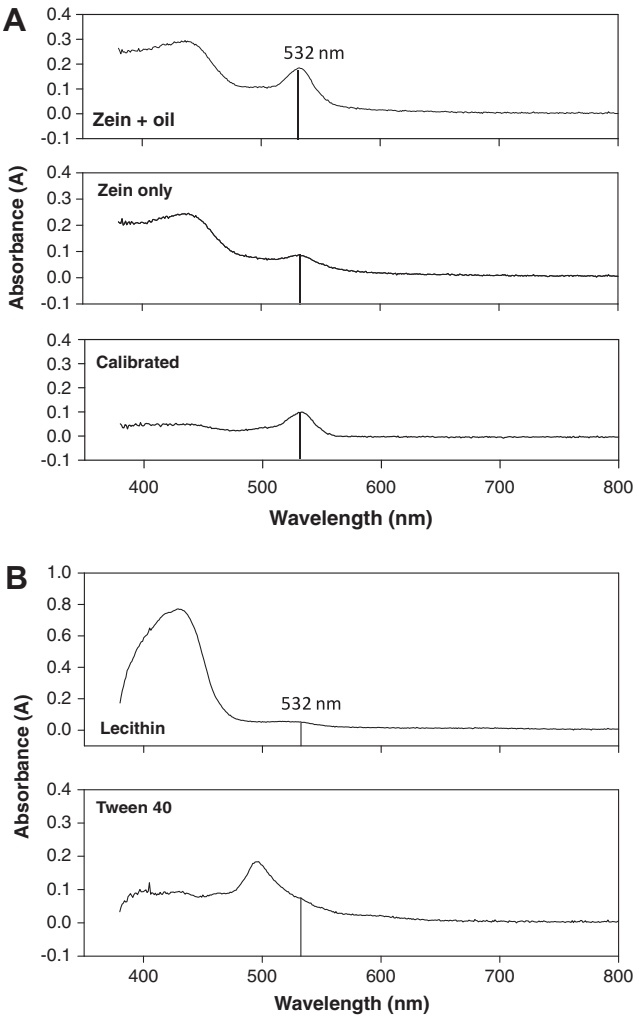

Fig. 2. Comparison of absorption spectra of the reaction products using: (A) zein capsules with ~20% fish oil (top curve) and zein alone (centre curve), calibrated by the TBA reagent blank; the bottom curve is plotted after calibration of the top curve by the centre curve and (B) 2 mg lecithin and Tween 40 in 10 mL TBA stock solution.

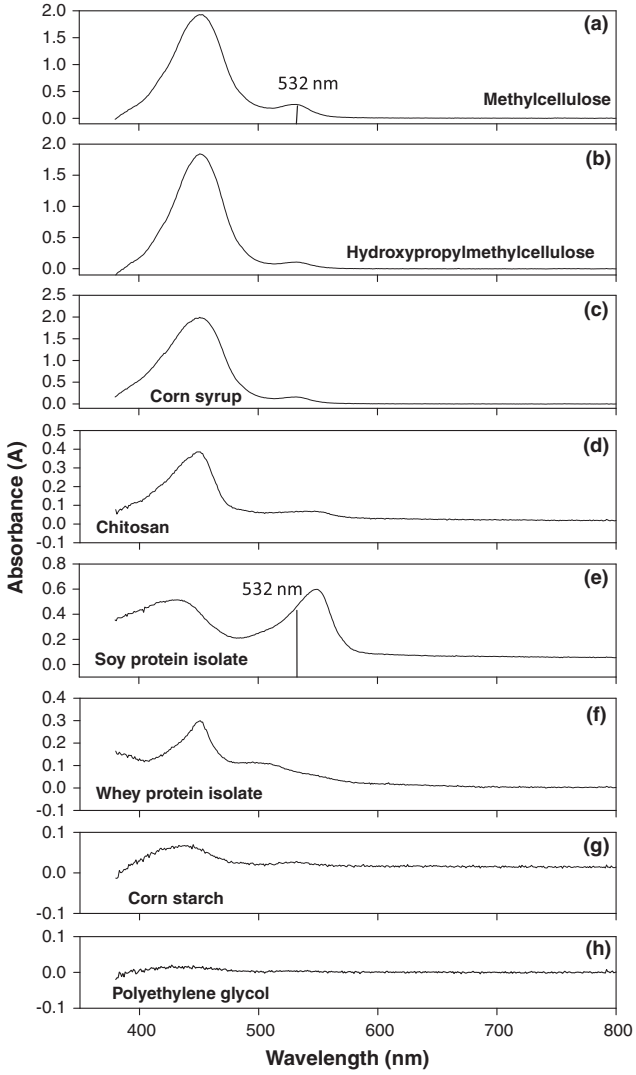

Fig. 3. Absorption spectra of TBA test products using 4 mg/mL common coating materials: (a) methylcellulose, (b) hydroxypropylmethylcellulose, (c) corn syrup, (d) chitosan, (e) soy protein isolate, (f) whey protein isolate, (g) corn starch and (h) polyethylene glycol.

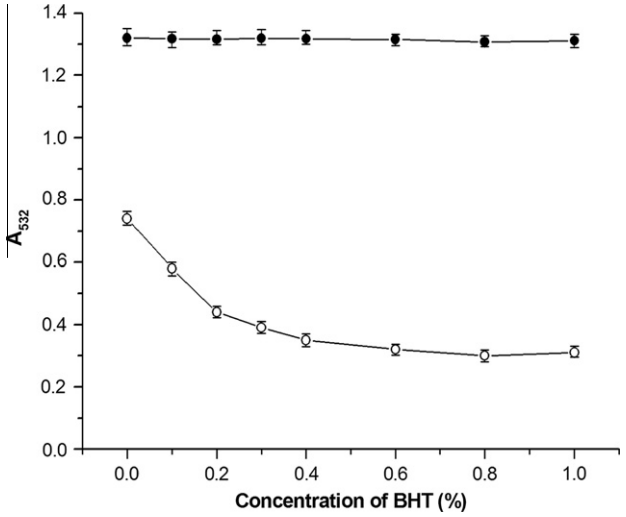

Fig. 4. Absorbance (at 532 nm, A_{532}) of reaction products when different concentrations of 2,6-di-tert-butyl-4-methylphenol (BHT) were used together with 8 μM TMP (●) or 4 mg/mL of the fish oil capsules (○) during TBA tests. Error bars represent standard deviations.

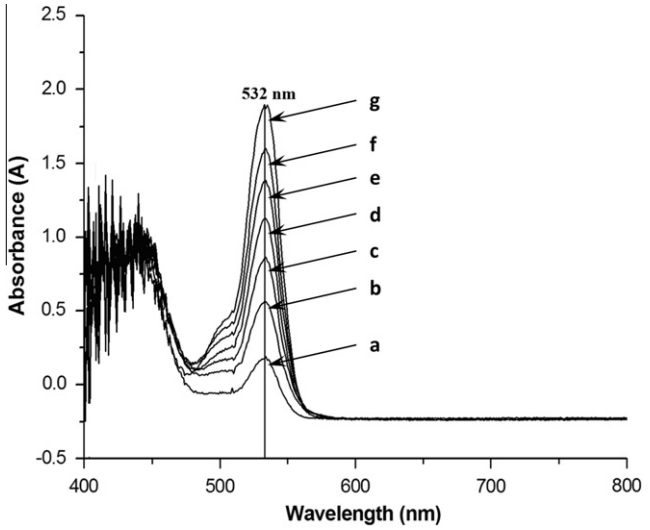

Fig. 5. Absorption spectra of the reaction products when different concentrations of 1,1,3,3-tetramethoxypropane (TMP) reacted with thiobarbituric acid (TBA), together with 3.1 mg/mL of zein and 0.8% w/v 2,6-di-tert-butyl-4-methylphenol (BHT). The curves represent systems with a TMP concentration of 2, 4, 6, 8, 12, and 16 μM, respectively, from the bottom to the top. The spectra were calibrated by the spectrum of the corresponding mixture without TMP.

to the above example of zein if SPI is used as a carrier material. Therefore, the selected ternary solvent system is able to dissolve a variety of carrier materials with a wide range of polarity, which may find many applications in relevant projects.

3.4. Effect of 2,6-di-tert-butyl-4-methylphenol (BHT) concentration in TBA tests

Because the high temperature and low pH during TBA tests may cause additional lipid peroxidation, antioxidants such as naturally-occurring tocopherols and synthetic BHT are used to reduce experimental errors due to TBA test procedures (Jentzsch, Bachmann, Furst, & Biesalski, 1996). In this work, we examined the effects of BHT concentration (0–1.0%, w/v in the TBA stock solution) on the assay results based on TMP (8 μM in the TBA stock solution) alone or the fish oil capsules (40 mg in 10 mL of the TBA stock solution). The results (Fig. 4) demonstrated that the addition of BHT did not change the absorbance reading of the TMP samples, as expected because TMP can not be oxidised further. Conversely, the absorbance values of the fish oil samples showed a dependence on the BHT concentration. The higher absorbance readings at a BHT concentration lower than ca. 0.6% may have been caused by lipid peroxidation during TBA tests. A constant absorbance at a BHT concentration higher than ca. 0.6% indicates minimised peroxidation during TBA tests. In experiments hereafter, the TBA stock solution was added with 0.8% (w/v) BHT during TBA tests.

3.5. Calibration curve for estimation of TBARS of fish oil capsules

Because of the significance of A_{532} (0.087) resulting from zein (Fig. 2A) and the need to include BHT in TBA tests to minimise lipid oxidation during assays (Fig. 4), a set of experiments were performed by dissolving 32 mg of zein in the TBA stock solution, together with 0.8% BHT and TMP at a concentration range identical to that in Fig. 1. The absorption spectra after calibration by that of the zein sample (without TMP) are presented in Fig. 5. The A_{532} was used to construct a second standard curve. After linear regression, the coefficient of determination (R^2) was 0.997, and the molar absorptivity was determined to be $1.33 \times 10^5\ M^{-1}\ cm^{-1}$, ~13% smaller than that based on TMP standard solutions without zein ($1.53 \times 10^5\ M^{-1}\ cm^{-1}$). The lowered sensitivity, as indicated by the lower calibrated A_{532} (Fig. 5 vs. 1) may have resulted from the binding of TMP with zein that resulted in lowered reactivity of TMP, but the examination of exact mechanisms is beyond the scope of this work.

Table 1
Evaluation of the accuracy and precision of the proposed assay system by spiking TMP in the assay solution with co-dissolved simulated fish oil capsules.

Sample	TMP added (μ mol)	Absorbance at 532 nm	Recovery (%)[c]	TBARS (mmol/kg oil)	RSD (%)[d]
Simulated capsules 1[a]	None	0.031 ± 0.001	–	0.291 ± 0.009	3.23
	0.01	0.159 ± 0.003	96.24	1.494 ± 0.028	1.89
	0.02	0.301 ± 0.003	101.5	2.829 ± 0.038	1.33
	0.03	0.435 ± 0.006	101.25	4.088 ± 0.056	1.38
Simulated capsules 2[b]	None	0.513 ± 0.007	–	4.821 ± 0.066	1.36
	0.01	0.651 ± 0.005	103.76	6.118 ± 0.056	0.92
	0.02	0.772 ± 0.008	97.37	7.256 ± 0.075	1.04
	0.03	0.921 ± 0.011	102.26	8.656 ± 0.103	1.19

[a] The simulated capsules 1 sample included a 40 mg mixture of zein, fish oil, and lecithin at a ratio of 20:5:1, where the fish oil was immediately used after thawing the frozen sample.
[b] The simulated capsules 2 sample was similar to the simulated capsules 1 sample except that fish oil was artificially oxidised by heating at 40 °C for 5 days.
[c] Recovery (%) was the ratio of TMP concentration estimated from the calibration curve to that actually used in TBA tests.
[d] Relative standard deviation (RSD) or coefficient of variation was the percentage of standard deviation over mean of TBARS.

The statistical analyses of the above two standard curves, i.e., from TMP standard solutions with or without zein, were compared after extending the TMP concentration range to 0.2–20 μM. When zein was absent, the linear range was observed for 0.2–16 μM TMP, which fell to 0.4–13 μM when zein was present. The slope, thus molar absorptivity, was higher for the TMP samples without zein (1.53 vs. 1.33 μM^{-1} for slope; 1.53×10^5 vs. $1.33 \times 10^5\ M^{-1}\ cm^{-1}$ for molar absorptivity). Within the linear range, both standard curves had a good linear regression (standard error < 4%, $R^2 \cong 1.000$, $p < 0.0001$).

3.6. Precision and accuracy

In order to evaluate the accuracy and precision of the TBA assay system, three levels of TMP were individually spiked into the TBA stock solution with a co-dissolved sample simulating fish oil capsules. The simulated fish oil capsules were composed of mixtures of zein, fish oil, and lecithin at a mass ratio of 20:5:1, similar to the formulation used in encapsulation by spray drying. In one sample, fish oil thawed from the frozen sample was used directly to simulate a lightly oxidised sample. In the other sample, the fish oil was artificially oxidised by heating at 40 °C for 5 days to simulate a moderately oxidised sample. The A_{532} readings and the estimated TBARS are listed in Table 1. The recovery, percentage of the TMP concentration estimated from the calibration curve to that spiked in the TBA stock solution, was close to 100% for all treatments, with an average recovery of 100.39%, indicating a good accuracy of the assay system. The relative standard deviation or coefficient of variation, i.e., percentage of standard deviation over mean, from five replicates of the estimated TBARS concentrations was all less than 3.3%, indicating a good precision of the assay system.

4. Conclusions

The ternary mixture of 1-butanol, isopropanol, and 0.5 M HCl (in water) at a proportion of 2:2:1 (v/v/v) was found to be able to dissolve fish oil as well appreciable amounts of several common encapsulation carrier materials. After reaction with TBA, samples with zein and SPI showed absorbance at the characteristic wavelength of TBA tests and needed a careful calibration. The proposed approach, by complete dissolution of encapsulation products, has the advantages of a small amount of test materials, the eliminated extraction or distillation step, good accuracy, sensitivity, and precision. The findings and the underlying principles may be applied for rapid and reliable assessment of lipid oxidation of various products.

Acknowledgements

This project was funded by the University of Tennessee Agricultural Experiment Station through a Hatch Project.

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