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ORIGINAL ARTICLE

CHARACTERIZATION OF SUNFLOWER, OLIVE AND MUSTARD OIL SURFACTIN BASED NANOEMULSIONS

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Abstract:

Selected cooking oils such as sunflower, castor, coconut, groundnut, and sesame oils were screened for the development of a surfactin based nanoemulsion formulation. Mean droplet size of different surfactin based cooking oil emulsions varied from 73.23 to 750.91 nm with the least size recorded by sunflower oil based nanoemulsion formulation. TEM analysis is used for to analysis of structure of AUSN-1 nanoemulson, HPLC method column used was a C18 column Phenomenex C18 250x4.6mm (5 micron). The mobile phase consisted of methanol, acetonitrile and water (v/v/, 66:32:2), at the flow rate and injection volume of 1.0ml/min and 20µl, respectivelly. The detection wavelength was 275nmwith UV detection at 275 nm. Retention time of the AT was found to be 4.044 min. was employed in this study. The Droplet size

KEYWORDS-

Characterization , sunflower , Nanoemulsions , biochemical.

INTRODUCTION

Nanoscience is defined as the study of phenomena and the manipulation of materials at the atomic, molecular and macromolecular scales, where the properties differ from those at a larger scale (Puglia et al., 2008). It is also explained as the control of matter on an atomic and molecular scale with at least one characteristic dimension measured in nanometer. Moreover, it is defined as the design, production and application of structures, devices and systems through control of the size and shape of the material at nanometer scale (Ramsden, 2005).

Nanotechnology tries to help this problem to measure, control and manipulate the matter at the nanoscale level to change those properties and functions in a beneficial way (Abbas et al., 2009). The discoveries made in nanotechnology also give impact to the food industry, due to the effect of biological and biochemical functionality of the system during the processing(Valdes et al., 2009).Besides that nanotechnology is also used as a means to understand how physicochemical characteristics of nano-sized substance can change the structure, texture and quality of foodstuffs (Chau, 2006). Many major areas in food production may benefit from nanotechnology, which is development of new functional materials, micro scale and nanoscale processing, product development and methods, instrumentation design for improved food safety and biosecurity, storage, transportation and traceability (ElAmin, 2007; Cobb and Macoubrie, 2004).

The rapid development in food industries improved tastes, color, flavor, texture and consistency of foodstuffs, increased absorption and bioavailability of nutrients and health supplements, new food packaging materials with improved mechanical, barrier and antimicrobial properties, nano-sensors for traceability and monitoring the condition of food during transport and storage (Ebbesen et al., 2006).

Nanoemulsion is an oil-in-water O/W type emulsion, with soybean oil, Triton X-100 and tributyl phosphate as non-aqueous components. Nanoemulsions represent the extremely small limit of emulsions of submicron droplets known as "miniemulsions" (Ugelstad et al., 1973, Tang et al., 1991) Although nanoemulsions have extreme Laplace pressures, of order 10–100 atm, the droplets can remain stable against Ostwald ripening if the liquid inside has very low solubility in the continuous phase outside the droplets. The strong Brownian motion of the tiny droplets in nanoemulsions makes them ideal for products in which gravitational creaming must be prevented to ensure a long shelf life (Tang et al., 1991).

Nanoemulsions are novel water-in-oil formulations that are stabilized by the addition of small amounts of surfactants (Baker et al., 2005). The water immiscible liquid phase is mixed into an aqueous phase by high stress mechanical extrusion, yielding a uniform population of droplets ranging in diameter from 400–800 nm. The droplets size and size distribution depend on the spontaneity of emulsification (Gopal, 1968; Becher, 1983; Shahizdadeh et al., 1999). The spontaneity of the emulsification is poorly defined, since it should account not only for the rate of the emulsification process, but also for the volume and the particle size distribution of the produced emulsion. The spontaneity of the emulsification process depends mainly on the following variables: interfacial tension, interfacial and bulk viscosity, phase transition region and surfactant structure and concentration (Lopes-Montilla et al., 2002).The structure of the nanoemulsion plays a vital role in its function (Florence, 1993; Gregoriadis and Florence, 1993; Wasan and Lopez-Berestein ,1995).

Nanoemulsions have long been employed in some applications, such as in detergents (Azemar, 1997), pharmaceuticals (Tenjarla, 1999; Lawrence and Rees, 2000) and cosmetics (Sonneville-Aubrun et al., 2004). The use of some fine emulsions as antimicrobials is a quite new and promising application. However, there is limited number of reports in the literature on their use for this purpose. Furthermore, there is a pressing need to develop novel strategies to combat the continuous increase in microbial resistance, due to the widespread, and sometimes inappropriate, use of antibiotics, disinfectants and antiseptics.The present study to investigate Properties of different oil based nanoemulsion. To analysis of sunflower based nanoemulsion structure using TEM

MATERIALS AND METHODS

Oils used for present study:

Sunflower oil (Helianthus annuus)
Olive oil(Olea europaea)
Mustard oil(Sinapis alba)

The oils were collected from the local market in Chidambaram, India.

Development of emulsions

The oil-in-water (O/W) nanoemulsion has an oil phase of sunflower oil (16% v/v of the total emulsion), ethanol (2%), and triton X-100 (2%). Therefore, this phase represents 20% (v/v) of the emulsion. The components of the oil phase were mixed and kept for 1 h at 86 °C. After this, the water phase was added, and the mixture was emulsified. Three oils, which include sunflower oil, coconut oil, and castor oil, were selected for further study.

Grading of emulsion

Grading of emulsion based on visual scoring method was carried out according to Zu¨ lli et al. 2006 to determine the degree of transparency, which is directly correlated to the size of emulsion. The emulsion was graded as A–F: A – rapidly forming (within 1 min) nanoemulsion, having a clear or slight bluish appearance; B – rapidly forming, slightly less clear nanoemulsion, having a bluish appearance; C – fine milky emulsion that formed within 2 min; D –dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min); E – formulation exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Yield of emulsion

The emulsion yield per 100 ml of oil and water phase was measured directly and expressed as yield in ml/100 ml.

Density

The density of a material is defined as its mass per unit volume. The hydrometer was used to measure density directly.

Refractive Index

Refractive index of selected formulations was determined using an Abbe type refractrometer. The viscosity of the prepared nanoemulsion formulations was determined as such without dilution by R/S CPS plus Rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using spindle # C 50-1 at 25±0.5 0C. The software used was RHEO3000. One ml of the formulation was used for viscosity determination. The speed of the spindle was adjusted to 70 rpm and a single run was performed at a temperature of 25±0.5 0C. Wait time for the operation was 50 min. Shear rate applied was 413 per min and diameter of the spindle used was 50 mm.

Stability

The stability was measured at constant temperature (250C) by multiple light scattering and dynamic light scattering. A turbiscan MA 2000 and a Malvern 4700 were used respectively.

Surface tension measurements

A series of protein solutions was prepared by dispersing different amounts of powdered protein (Onsaard et al., 2006) into buffer solution (5 mM phosphate buffer, pH 7), followed by stirring at 4°C overnight. Surface tension measurements were conducted using a digital tensiometer (K10 tensiometer, Kruss Scientific, Hamburg, Germany) equipped with a roughened platinum Wilhelmy plate. The platinum plate used in the experiments was cleaned and flamed in a Bunsen burner prior to each experiment. Protein solution (40 mL) was poured into the measurement vessel of the tensiometer and allowed to equilibrate to the measurement temperature (30.0 0C) for 20 min. The surface pressure (p) was calculated from p = c0 _ c, where c0 and c are the surface tensions of pure buffer solution (71.05 mN/ m) and protein in buffer solution, respectively.

Viscosity

The viscosity of the various nanoemulsion preparation was measured using an ubbelhode viscometer (VBB-OB, VWR international, MA, USA) and used for particle size measurement. The suspension of aspirin had a viscosity of 0.98 cP white blank and nanoemulsion of aspirin had a viscosity of 2.16 cP at room temperature.

HPLC analysis in AUSN -1

The HPLC equipment consisted of quaternary LC-10A VP pump, SPD-10AVP column oven, variable wavelength programmable UV/VIS detector, SCL 10AVP system controller, Rheodyne injector fitted with a 20 µL loop, degasser and a data processor all from Shimadzu, Kyoto, Japan. Class-VP 5.032 software was used to record and evaluate the data collected during and following chromatographic analysis.

Transmission Electronic Microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using Transmission Electron Microscopy (TEM) LEO 912AB EFTEM. To perform the TEM observations, samples were placed on a formvar carbon-coated copper grid (200 mesh in-1) and then stained with 1% phosphotungstic acid. The excess phosphotungstic acid on the sample was gently wiped off using filter paper and examined after drying for about half an hour at room temperature.

EXPERIMENTAL RESULTS

Three oils were selected screened for nanoemulsion formulation and the results are present in table 1. The higher yield occurs was observed in sunflower oil (34.21%), followed by olive oil (15.20%), mustard oil (10.54%).Based on % of yield from the three different screened nanoemulsion formulation oils such as sunflower oil selected for further studies.

These emulsions were studied for different physiological properties such as Surface tension reduction (N/m), Viscosity (Nsm-2), Stability, Density and Refraction index respectively. They were also further characterized as winsor class I, II and III according to the standard procedures and the surfactant yield per 100ml and stability was also estimated and the results are presented in table 1.The emulsion, which showed a higher degree of transparency and according winsor class (class I), sunflower oil based emulsion (referred as nanoemulsion) was selected for further study.

Among the different oils tried; surfactin based sunflower oil nanoemulsion was found to Surface tension 0.032 N/m,GradeA, Viscosity 19.032 Nsm-2, Stability ++, Density 692,103Kgm-3 , Refractive index 1.219 followed by olive oil and mustard oil. Earlier studies (Zu¨ lli et al 2006) reported that nanoemulsions are transparent (translucent) dispersions of oil and water having a droplet size of less than 100 nm and found to be thermodynamically stable. It is the thermodynamic nature that makes them stable and not subject to phase separation, creaming,or cracking (Shinoda, 1983)

Table1. Shows the viscosity (19.032 to 19.245) of the formulation was observed. As the volume of dispersed phase increased viscosity the density also increased. Overall, very low viscosity of the nanoemulsion formulations was in sunflower oil. Joe et al 2012 reported AUSN-1 recorded the least surface tension, viscosity, and density. We suppose that this property of sunflower oil based nanoemulsion is attributed to the lower fat (saturated and monounsaturated) content of sunflower oil, when compared to the other oils evaluated.

Nanoemulsion formulation during storage at 4 °C presented in table 2.The droplet size, viscosity, Density, Refractive index and Surface tension of sunflower oil nanoemulsion formulation were not significantly changed during 3 months of storage at 4°C period suggesting the physical stability of the prepared nanoemulsion. The changes in these parameters were not statistically significant ($P \geq 0.05$). These results indicated that the optimized formulation was stable as there were no significant changes in physical parameters (droplet size, viscosity Density, Refractive index and Surface tension and). Sarfaraz et al 2012 reported these parameters were determined at 0, 1, 2 and 3 months. It was found that at 4 and 250C these parameters were slightly increased during storage at both the temperatures

HPLC analysis of AUSN1 shows fig1 .The HPLC column used was a C18 column Phenomenex C18 250x4.6mm (5 micron). The mobile phase consisted of methanol, acetonitrile and water (v/v/, 66:32:2), at the flow rate and injection volume of 1.0ml/min and 20µl, respectivelly. The detection wave length was 275nm. All operations were carried out at room temperature. The calibration curve of vitamin A palmitate was constructed. The precision, reproduction and percent recovery of the method were analyzed. The validated method was applied for determination of AT 0.044. This method is based on the previously reported procedures (Nirogi et al., 2007, Bahrami et al., 2005., Gulam Mustafa et al 2010)

In order to observe the physical properties of the oil droplets in the nanoemulsion sample, TEM analysis was performed with negatively stained samples. Figure 2b shows the TEM images for the nanoemulsion sample water in oil nanoemulsion was used, the spherical shape of the nanoemulsion became more regular and smaller droplets were dominant. The droplet size range of 73.23-750.91 nm was obtained and revealed good size distribution.

CONCLUSION

Sunflower oil based Nanoemulsions considered in this review represent a special class of liquid disperse systems. Their droplet sizes are less than 100 nm but the systems are kinetically stable compared to olive oil and mustard oil. Despite thermodynamic instability, they can exist for a rather long time (a few months). Its contain antibacterial and fungi static property

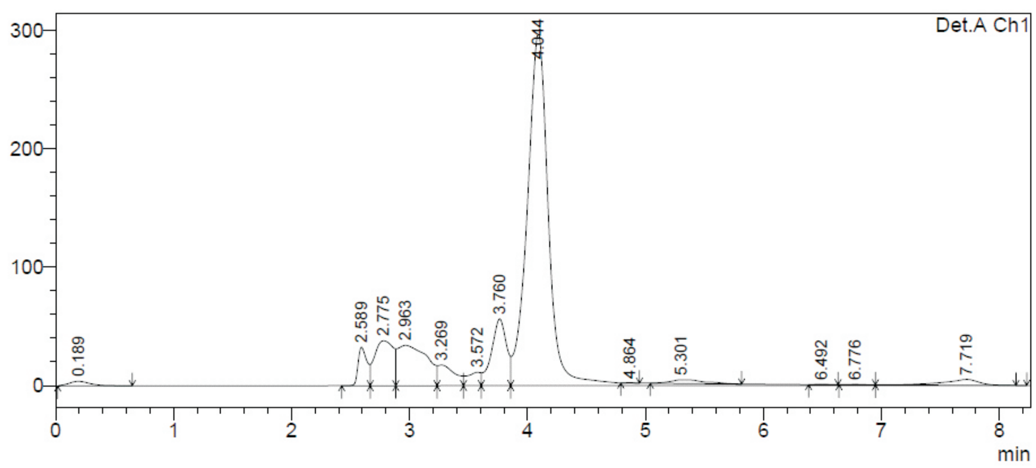
Table 1Properties of sunflower, olive and mustard oil surfactin based cooking oil emulsions.

Oils	Surfactant	Winsor class	Yield ml/100ml	Mean droplet size	Surface tension N/m	Grade	Viscosity Nsm^{-2}	Stability	Density Kgm^{-3}	Refractive index
Sunflower Oil	Triton X-100	I	34.21±0.04	73.23±0.00	0.032	A	19.032±0.42	++	692.03±0.23	1.21±0.17
	SDS	I	32.41±0.12	138.44±0.32	0.034	B	19.063±0.08	++	714.89±0.17	1.22±0.03
Olive oil	Triton X-100	I	15.20±0.01	320.11±0.17	0.042	B	19.093±0.03	++	782.71±0.08	1.29±0.01
	SDS	II	12.43±0.03	370.45±0.23	0.045	D	19.099±0.23	+-	832.24±0.17	1.29±0.23
Mustard Oil	Triton X-100	II	10.54±0.12	688.67±0.03	0.044	E	19.240±0.00	-	871.64±0.12	1.32±0.42
	SDS	III	9.16±0.14	750.91±0.08	0.049	E	19.245±0.12	-	884.64±0.02	1.37±0.08

TABLE 2 Droplet size, viscosity, Density Refractive index and surface tension of AUSN-1 nanoemulsion formulation during storage at 4^{0}C

Time (Months)	Droplet size	Viscosity	Density	Refractive index	Surface tension
0	73.23±0.0	19.032±0.42	692.03±0.23	1.21±0.17	0.032
1	73.21±0.0	19.032±0.42	692.02±0.23	1.20±0.17	0.032
2	73.18±0.12	19.025±0.33	691.97±0.23	1.17±0.14	0.030
3	73.10±0.04	19.018±0.22	691.90±0.23	1.15±0.00	0.027

Fig 1 HPLC chromatograms obtained from the analysis of AUSN1



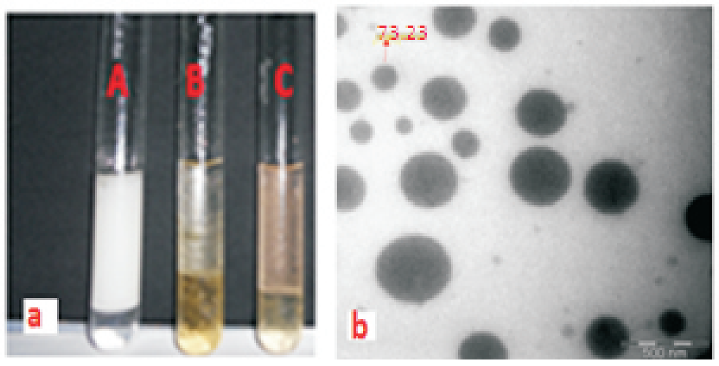


Fig2a. Development of different surfactin based emulsion from (A) Mustard Oil, (B) sunflower, (C) olive oil, b) TEM analysis of sunflower oil based Nanoemulsion AUSN-1

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