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Evaluation of Different Methods for Preparing Nanoparticle Containing Gammaoryzanol for Potential Use in Food Fortification

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ABSTRACT

Background: Gammaoryzanol is a natural antioxidant and could provide beneficial as used in the food products due to the antioxidant activity and potential health benefits. Nanotechnology has been introduced into several aspects of the food science, including encapsulation of materials and used as delivery systems. The field of nanoparticle delivery systems for nutrients and nutraceuticals has been expanding over the last decades. ***Purpose:*** The aim of this work was evaluation of different methods for preparation of polymeric nanoparticle containing gammaoryzanol. ***Methods:*** Nanoprecipitation technique, where polymer and gammaoryzanol were dissolved in acetone, nano-emulsion template method, by stepwise addition of water in to the oil phase consisting of ethyl acetate, gammaoryzanol and surfactant mixtures, as well as emulsification solvent evaporation technique, where gammaoryzanol and polymer were dissolved in ethyl acetate and chloroform, were used for preparation of nanoparticles. Two ratio of gammaoryzanol-polymer (Ethyl cellulose) (1:2 and 1:4) as well as different solvents and surfactants were used in these methods to produce gammaoryzanol nanoparticles. ***Results:*** Among these methods, solvent evaporation technique has been successfully employed to produce gammaoryzanol loaded nanoparticles with desired characteristics.

Introduction

Gammaoryzanol is one of the natural antioxidants where extracted from the rice bran oil that possess many applications in food products and medicine.[1] It is a mixture of ferulic acid esters of sterol and triterpene alcohols.[2] Gammaoryzanol has high potential as a functional food ingredient and as a stabilizer of vegetable oils and fats at elevated temperatures.[3] In addition, it has unique properties and many health beneficial properties including reduction of total cholesterol, improvement of the plasma lipid pattern and inhibition of platelet aggregation.[4,5] Moreover, it has been proposed as a UV-A filter in sunscreen cosmetics[6]. Because of its poor water solubility, the application of gammaoryzanol in food products is limited and its intestinal absorption and bioavailability is very poor.[3]

Nanotechnology has been introduced into several aspects of the food science, including encapsulations and delivery systems,[7] which has versatile advantages such as incorporation of the bioactive compounds into the food matrices with high physicochemical stability and minimal impact on the properties of the product,[8] as well as protection of the encapsulated bioactive compounds from the interaction with other food components,[9] and maximize the uptake of the encapsulated compounds upon intake and their transport to the sites of action.[10] Nanoparticles are one of the different types of nano-sized carriers generally have size below than 1000 nm that being developed for drug delivery applications.[11] Nanoparticles classified as non-degradable and biodegradable.[12] In recent years, there has been considerable attention to developing biodegradable nanoparticles due to their higher encapsulation efficiency, controlled release and less toxic properties.[13,14] Numerous processes have been extensively described in past years for the preparation of nanoparticles, including emulsion/solvent evaporation,[15] nanoprecipitation,[16] emulsion and miniemulsion polymerization,[17] salting out,[18] using supercritical fluid technology,[19] electrohydrodynamic atomization,[20] and the generation of nanoparticles using the nano-emulsion template.[21]

The nanoprecipitation technique (or solvent displacement method) is a modified solvent evaporation method for nanoparticle production. In this method the water-miscible solvents like acetone or methanol along with the water immiscible organic solvent like chloroform or dichloromethane were used as an oil phase. Rapid dispersion of the polymers in the presence of water can cause precipitation of polymers. This technique has many advantages like rapid and easy to perform where the entire procedure could carried out in only one step.[22-24]

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Nano-emulsions are emulsions with droplet sizes usually in the range of 20–500 nm. Nano-emulsions can be achieved by both high-energy and low-energy methods. Low-energy methods produce smaller and more uniform droplets. The obtained nano-emulsions are prepared by phase inversion temperature or phase inversion composition methods.[21,25-27]

In the emulsion solvent evaporation technique the polymer is dissolved in an organic solvent like dichloromethane, chloroform and ethyl acetate and the drug compound is dissolved or dispersed in the polymer solution. The mixture is emulsified in an aqueous solution containing surfactant and finally emulsified by high-energy homogenizer, resulting in producing oil in water emulsion. Being simple, fast and economical as well as having the benefit of employing non-toxic solvents are advantages of using this method.[28-30]

In the present research, we compared emulsion solvent evaporation and nanoprecipitation techniques as well as the use of nano-emulsion by low-energy method to produce polymeric gammaoryzanol-loaded nanoparticles with desired characteristics, to be used as an antioxidant and nutraceutical compound in the food stuffs.

Materials and methods

Materials

Gammaoryzanol (GO) was received as a gift from Tsuno Rice Fine Chemicals Company. (Japan). Ethyl Cellulose (EC) and Poly vinyl alcohol (PVA) were obtained from Sigma Company (Germany). Tween 80 was purchased from Merck Company and tween 20 was provided by Scharlau Company (Germany). Water was obtained from a Milli-Q Plus purification system and all other used chemicals and solvents were of analytical grade.

Nanoprecipitation technique

Ethyl Cellulose and gammaoryzanol were dissolved in acetone as a water-miscible organic solvent to form the diffusing phase. Diffusing phase (15 mL) was added to the dispersing phase (35 mL) containing surfactant (PVA, 1 % w/v) by using a syringe under high speed homogenizer (20000 rpm) for 5 minutes. Formulations were prepared with two different ratios of GO:EC (Table 1). All the formulations were prepared in twice to get the reproducibility and reliability.

Table 1. Formulations prepared by nanoprecipitation technique

Formulation Code	GO:EC (w/w)
F1	1:2
F2	1:4

GO: Gammaoryzanol, EC: Ethyl cellulose

Nano-emulsion template

Emulsions were prepared by stepwise addition of water (15 mL) to organic_phase (35 mL, 10 % EC in ethyl acetate and surfactant mixture) at 25 ºC under moderate magnetic stirring (600 rpm). Then particles were prepared by overnight evaporation of the solvent presented in the emulsion under continuous stirring at room temperature.

Ratio of organic phase–surfactant in all of formulation was 50:50. Two types of surfactants and two different ratios of GO:EC were used for preparation of formulations (Table 2).

Table 2. Formulations prepared by nano-emulsion template

Formulation Code	GO:EC (w/w)	Surfactant type
F3	1:2	Tween 20
F4	1:4	Tween 20
F5	1:2	Tween 80
F6	1:4	Tween 80
F7	1:2	Tween 80 (25%) + Tween 20 (75%)
F8	1:4	Tween 80 (25%) + Tween 20 (75%)

GO: Gammaoryzanol and EC: Ethyl cellulose

Emulsion solvent evaporation technique

An adequate amount of gammaoryzanol and EC were dissolved in 15 mL of ethyl acetate and chloroform as organic phase at room temperature using a magnetic stirrer.

Table 3. Formulations prepared by emulsion solvent evaporation technique

Formulation Code	GO:EC (w/w)	Solvent type	Sonication
F9	1:2	chloroform	_
F10	1:4	chloroform	_
F11	1:2	ethyl acetate	_
F12	1:4	ethyl acetate	_
F13	1:2	chloroform	+
F14	1:4	chloroform	+
F15	1:2	ethyl acetate	+
F16	1:4	ethyl acetate	+

GO: Gammaoryzanol, EC: Ethyl cellulose, -: without sonication and +: with sonication

The aqueous phase of PVA (20 mL) was added dropwise using a plastic syringe (35 mL) with 22 gauge

needle to the organic phase and homogenized using homogenizer (Heidolf, Germany) at 20,000 rpm for 15 minutes followed by sonication using probe sonicator (Hielscher, UP200S, Germany, 70 % of maximum intensity, 5 times with 1 min on and 1 min off) in an ice bath. The organic solvent in emulsion was removed by overnight evaporation at room temperature under continuous stirring to obtain nanoparticles. All the formulations with two different ratio of gammaoryzanol and ethyl cellulose were prepared in triplicate (Table 3).

Size determination

A laser light scattering particle size analyzer (SALD 2101, Shimadzu, Japan) was used to determine the particle size of the nanoparticle formulation. Samples were suspended in distilled water and stirred continuously during the particle size analysis.

The size distribution was expressed by the volume median diameter (VMD) and span value. Span is a measure of the width of the size distribution. Where D (v,90), D (v,10) and D (v,50) are the equivalent volume diameters at 90%, 10% and 50% cumulative volume, respectively [31].

$$\textbf{Span} = \frac{\mathbf{D(v,90)-D(v,10)}}{\mathbf{D(v,50)}} \qquad \text{Eq. (1)}$$

Results and discussion

Nanoprecipitation technique

Average particle diameter of polymeric particles were determined by dynamic light scattering method and results were listed at Table 4. Both prepared formulations (F1 and F2) using this method possess the micron size range. Nanoprecipitation method often enables to produce small nanoparticles with narrow distribution,[22] however, producing gammaoryzanol loaded nanoparticle using surfactant with these used concentrations and the GO:EC ratio of 1:4 could not be achieved by this method.

Table 4. Mean diameter and span value of particles prepared by nanoprecipitation technique

Formulation Code	Size (nm±SD)	SPAN
F1	2495.5±4.95	3.19±3.60
F2	2806.5±23.33	4.60±0.50

SD: Standard deviation

Nano-emulsion template

The average diameter and size distribution of formulations prepared by nano-emulsion template are shown in Table 5. The average diameter of particles were in the micron size range in all of the formulations. Increasing GO:polymer ratio and use of different type of surfactants, could not decrease the size of particles up to nanometer range. These results are inconsistent with the results obtained from Calderó et al investigation.[21]

Table 5. Mean diameter and span value of particles prepared by nano-emulsion template.

Formulation Code	Size (nm±SD)	SPAN
F3	2022±19.79	1.34±0.09
F4	2079.5±20.50	1.39±0.19
F5	1894±12±73	0.88±0.09
F6	1978.5±9.19	0.55±0.11
F7	1430±22.63	0.65±0.16
F8	1473.5±32.50	0.49±0.030

SD: Standard deviation

Emulsion solvent evaporation technique

The average diameter and Span of formulations prepared by two used solvents (chloroform and ethyl acetate) with or without sonication treatment are shown in Table 6. Use of chloroform without sonication produced particles with size range over than 1000 nm (F11 and F12). However, use of probe sonication for 5 min reduced particles size up to 750 nm.

Table 6. Mean diameter and span value of particles prepared by emulsion solvent evaporation technique.

Formulation Code	Size (nm± SD)	SPAN
F9	1496±62.93	0.45±0.02
F10	1473±43.84	0.48±0.02
F11	831±50.91	1.07±0.26
F12	783±21.21	0.94±0.15
F13	712.5±14.85	0.68±0.11
F14	726±2.82	1.16±0.01
F15	94.5±3.53	0.55± 0.64
F16	81±4.24	0.88± 0.08

SD: Standard deviation

Use of ethyl acetate as solvent produced particles with smaller size than particles prepared using chloroform. In this method, emulsions were formulated with

polymer solution in volatile solvents. Dichloromethane and chloroform were widely used in the previous investigations, but nowadays were replaced with ethyl acetate which displays a better toxicological profile.[2]
Ethyl acetate is a Generally Recognized as Safe (GRAS) solvent with low toxicity (median lethal dose, LD50, in rats: 11.3 g/kg).[32] Therefore, it was safely used as a suitable solvent to produce gammaoryzanol nanoparticle.
Formulations F15 and F16 showed particle size below than 100 nm. In this formulations ethyl acetate solvent and ultrasonication treatment were employed.

Particle size and size distribution is an important criteria of nanoparticles as this factors affect most of biological actions attributed to the size of particles such as drug release rate, biodistribution, mucoadhesion, cellular uptake, where smaller size showed higher intracellular uptake than particles with larger size range.[29,33]

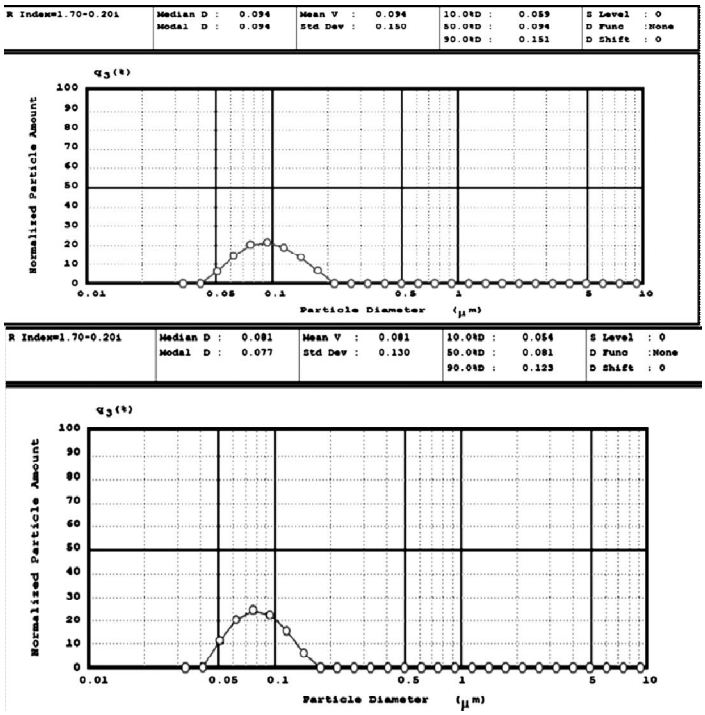

Figure 1. Particle size distribution of nanoparticles [formulation F15 (top) and F16 (below)]

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Conflict of interest

The authors declared that they have no conflict of interest.

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