JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2020, *26*(3), 140-148

Journal of Agroalimentary Processes and Technologies

Fortification of pear juice with vitamin D3 encapsulated in polymer microparticles: physico-chemical and microbiolgical characterization

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Abstract

The objective of this study was the fortification of pear juice with vitamin D3 loaded in polymer microparticles. Vitamin D3 loaded in gum arabic-chitosan microparticles were prepared using a spray drying technique. The pear juice fortified with vitamin D3 was ultrasonicated to increase stability and shelf life. Vitamin D3 loaded in gum arabic-chitosan microparticles with an average diameter (d_{43}) of 12.64±1.14µm and encapsulation efficiency of 89.78±3.88% were prepared and used to fortification. After ultrasonication of fortified pear juice, the polyphenol content (~0.18mg GAE/mL), flavonoid content (~0.05mgCE/mL) and vitamin D3 content have undergone insignificant changes ($p>0.05$), while antioxidant activity increased and the total number of mesophilic aerobic bacteria decreased. The investigated quality parameters of pear juice fortified with vitamin D3-loaded in gum arabic-chitosan microparticles remained unchanged after seven days of storage at 4ºC.

Keywords: vitamin D3, spray drying, polymer microparticles, fortification, sonication, pear juice, physico-chemical and microbiological characterization

1.Introduction

Vitamin D is a micronutrient with major implications for human health [1]. Therefore, in the last decade, more than 80,000 papers have appeared in the literature that have included studies on the mechanisms of the physiological processes of vitamin D, the stability and bioavailability of vitamin D and on the techniques of fortifying foods with vitamin D [2, 3, 4, 5]. A low vitamin D content in the daily intake causes imbalances in the body, such as calcium-phosphorus imbalance and parathyroid imbalance [4]. Research has shown that vitamin D plays an important role in preventing diseases such as osteoporosis, diabetes, cancer, cardiovascular disease, immunological diseases, etc. [6]. Vitamin D exists in two forms: vitamin D2 (ergocalciferol) not synthesized by the human body and vitamin D3 (cholecalciferol) synthesized by the human body on the skin through processes stimulated by UV radiation.

Researchers have shown that vitamin D3 is more active than vitamin D2 in the body's metabolic processes [7]. It has been found that the content of vitamin D3 synthesized by the human body is not sufficient to meet the requirements of biological processes. There are also few natural sources rich in vitamin D. Therefore, many countries have allowed the fortification of foods with vitamin D, such as edible oil, margarine, milk, dairy products, bread, beverages etc. [4, 8]. Today, more and more people prefer fruit juices due to the high content of bioactive compounds that contribute to human health [9].

Pears are the favorite fruit of consumers of all ages due to their pleasant taste and high nutritional value. Consumption of fresh pears or pear juice brings a high intake of vitamins, minerals, carbohydrates and phenolic compounds [10, 11].

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The low content of vitamin D in pear juice can be improved by fortifying it. Due to the high consumption of pear juice, by fortification with vitamin D, it could become an important vector for vitamin D distribution, especially in children, to prevent vitamin D deficiency. Adding fat-soluble vitamins, A, D, E, K, to fruit juices is a real challenge for producers [12]. To this end, many researchers studied the fortification of fruit juices with different fat-soluble vitamin delivery systems, such as: emulsions [13, 14], double emulsions [3], liposomes [15], polymeric particles [16].

This paper investigated the fortification of pear juice with vitamin D3 encapsulated in polymer microparticles prepared by the spray-drying method. Two food-grade natural biopolymers such as gum arabic and chitosan were used as encapsulating material. Gum arabic is an anionic polysaccharide that contains amino acid residues in its structure [17]. Due to its water solubility and surfactant properties, gum arabic has been used extensively in encapsulating bioactive compounds by the spray drying method [18]. Chitosan is a natural compound (aminopolysaccharide with a linear structure) with special properties: it is biocompatible, biodegradable, bioadhesive and biologically active (antibacterial, anti-inflammatory, mucoadhesion properties) [19]. At low pH values, chitosan has a polycation structure due to the protonation of amino groups. Therefore, in the presence of gum arabic or other polyanions, it forms nanoparticles by the complex coacervate method [20].

Several physicochemical analyzes of fortified pear juice with vitamin D3 entrapped into polymer microparticles, such as total soluble solids (ºBrix), pH, titrable acidity, vitamin C content, vitamin D content, total phenolic content, total flavonoids content, antioxidant activity, and microbiological assays, have been performed.

2.Materials and methods

Vitamin D3 (VD3) (cholecalciferol crystalline 98%, CAS 67-97-0), gum arabic (GA) (CAS 9000-01-5), chitosan (Ch) (CAS 9012-76-4) and polyoxymethil sorbitan monooleate Tween 80 (CAS 9005-65-6) were purchased from Sigma-Aldrich (St. Louis, MO, SUA). Linseed oil (67.85% polyunsaturated fatty acids) was purchased from Solaris Plant SRL (Romania). The pears (*Pyrus bretschneideri Rehd*) were purchased from the local supermarket. All the other reagents used in the experiments were of analytical grade.

2.1. Preparation and characterization of VD3-GA/Ch microparticles

For the encapsulation of VD3 in gum arabic-chitosan microparticles, the spray drying method was used Dima *et al.*, (2016) [21]. The process was done in two steps. In the first step, an Oil-in-Water (O/W) emulsion was prepared to contain the oily phase and the aqueous phase in a 1: 4 mass ratios. Linseed oil with VD3 (9:1 w/w) was used as the oily phase. The aqueous phase contained: 16% (w/w) polymers (GA: Ch, 9:1w/w) and 1.5% (w/w) Tween 80. The mixture was sonicated, at an amplitude of 40%, for 5 min (Sonoplus, Bandelin, Germany, platinum horn of 3 mm diameter). The temperature was maintained constant at 25º C. In the second step, VD3-loaded microparticles was done by spray drying technique using Buchi mini spray dryer apparatus (Model B-191, Buchi Laboratorium-Technik, Germany). Spray drying parameters were: aspiration rate 45%, atomization air pressure 3 kg/cm^2, a feed rate of 5 mL/min, T_{inlet} and T_{outlet} were 180ºC and 80ºC, respectively. The obtained powder was weighed and sealed into dark plastic bottles and kept at room temperature for analysis.

The encapsulation yield (EY) was calculated with the equation (1):

$$EY = \frac{m_2}{m_1} \times 100 \qquad (1)$$

where: m_2 is the mass of powder obtained in the collecting vessel; m_1 is the total mass of the oil-in-water emulsion [22].

The moisture content (MC) of the powder was determined by the gravimetric method according to the AOAC (2007) [23] and was calculated with the equation (2):

$$MC = \frac{m}{m_0} \times 100 \qquad (2)$$

where: m_0 is the initial mass of weighed powder (1g); m is the final mass of the dry powder.

The size of the VD3-GA/Ch microparticles was measured by the light scattering technique using a particles analyzer (PA-200G, mrc Laboratory Equipment Ltd, Israel). The samples were suspended in isooctane and the volume-weighted mean diameter ($d_{4,3}$) and SPAN factor were measured. SPAN factor calculated with equation (3):

$$SPAN = [D(v, 90) - D(v, 10)]/D(v, 50) \quad (3)$$

where: D (v, 90), D (v, 10) and D (v, 50) are volume size diameters a 90,10 and 50% of the cumulative volume, respectively.

The morphology of the VD3-loaded microparticles was analyzed using a Scanning Electron Microscop (SEM) (Quanta 200 FEI, Netherlands), with a magnification between 500 and 9.000x. The samples were fixed directly on aluminium stubs using electrically conductive double adhesive tape (Agar Scientific, Christine Gropl Austria).

The encapsulation efficiency (EE) was determined using a modified method described by Lin *et al.*, (2016) [24]. 100 mg of dry powder was added to 5 ml mixture of isooctane and ethyl alcohol (1:3 w/w) to remove the free vitamin (encapsulated VD3). The suspension was shaken for 5 min, then was filtered using a Whatman No. 1 filter paper. The residue was subjected to this procedure twice. The three filtrates were combined and the absorbance was measured at 264 nm (UV-VIS spectrophotometer, Jasco 560, Germany). To determine the VD_3 content, the regression equation was obtained ($y = 0.1279x + 0.0692$; $R^2 = 0.9946$), using the standard solutions of VD3 in isooctane/ethyl alcohol (5-20 µg/mL). The encapsulation efficiency was expressed as a percentage of vitamin entrapped and calculated with equation (4):

$$EE = \frac{W_0 - W_f}{W_0} \times 100 \quad (4)$$

where: W_0 is the weight of formulated VD3 and W_f is the weight of free VD3

2.2. Preparation and characterization of pear juice fortified with VD3-GA/Ch microparticles

Preparatory to obtain the juice, the pears (*Pyrus bretschneideri Rehd.*) were washed and cleaned, the seeds were removed and then cut with a knife into four parts. The pear juice was obtained by cold pressing using an extractor Panasonic MJ-M176P. After extraction, the pear juice was filtered to obtain a clear sediment-free juice. Fortification with VD3-GA/Ch microparticles was performed before and after the ultrasound process using Sonoplus Bandelin, Germany, under the following conditions: amplitude 40 KHz; temperature 40ºC for 15 min. After the ultrasound, the samples were sealed into sterilized glass bottles and kept at 4ºC.

2.3. *Physico-chemical characterization of pear juice fortified with VD3-GA/Ch microparticles*

The total soluble solids were performed using the refractometer RL-3 (PZO, Warsaw, Poland) and expressed in ºBrix; *p*H was performed using the multi-parameter analyzer Consort C862 equipped with *p*H electrode (HI 1131B); titrable acidity was performed using the standard method AOAC, (1999) [25].

The vitamin C content was determined using the iodometric method. Vitamin C extracted from pear juice with HCl (2%) was titrated with potassium iodate in the presence of potassium iodide and starch until blue color. The vitamin C content was expressed as mg ascorbic acid (AA)/100 ml pear juice AOAC, (1999) [25].

The VD3 content of pear juice before and after the fortification and ultrasound process was determined using the same method used to measure VD3 content from GA/Ch microparticles [24].

The total phenolic compounds (TPC) were determined using the Folin-Ciocalteu method Horincar *et al.*, (2019) [26]. 200 µL of pear juice were diluted in distilled water (15.8 mL), which was added 1 mL of Folin-Ciocalteu reagent. After 10 minutes, 3 mL of sodium carbonate solution (20%) was added and after 60 minutes (when the mixture was kept in the dark conditions), the absorbance was measured at 765 nm. The TPC was expressed as mg gallic acid equivalent (GAE)/mL pear juice (mg GAE/mL).

The total flavonoids content (TFC) was determined using the colorimetric method described by Horincar *et al.*, (2019) [26]. In 0.25 mL pear juice, 1.25 mL distilled water and 0.75 mL sodium nitrite solution (5%) were added. The mixture was kept at room temperature for 5 minutes, then 0.15 mL aluminum chloride solution (10%), 0.5 mL sodium hydroxide 1M and 0.75 mL distilled water were added. The absorbance was measured at 510 nm and TFC was expressed as mg catechin equivalents (CE)/mL pear juice (mg CE/mL).

Antioxidant activity of pear juice was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, according to previously described protocol Nowak and Groslinski, (2020) [27] with modifications. A 0.1 mL of pear juice was added to 3.9 mL DPPH solution and mixed.

The mixture was kept for 90 minutes at room temperature in the dark. The absorbance was measured at 515 nm. The antioxidant activity was expressed as mMol Trolox/ml pear juice using a calibration curve.

2.4. Microbiological characterization of pear juice fortified with VD_3-GA/Ch microparticles

The microbiological characterization was achieved by performing the following analyzes:

- Horizontal method for the enumeration of microorganism (mesophilic aerobic bacteria) (ISO 4833-2:2013) expressed as colony forming units (CFU)/mL pear juice, (CFU/mL) [28];
- Horizontal method for the detection and enumeration of *Enterobacteriaceae* (ISO 21528-2:2007) expressed as colony-forming units/mL pear juice, (CFU/mL) [29];
- Horizontal method for the enumeration of yeasts and moulds (ISO 21527-2:2009) expressed as colony-forming units/mL pear juice (CFU/mL) [30].

2.5. Statistical analyses

All experiments in this study were performed in triplicate and for statistical calculation, the one-way analysis of variance (ANOVA) with Tukey's test ($p \leq 0.05$) were used.

3. Results and discussion

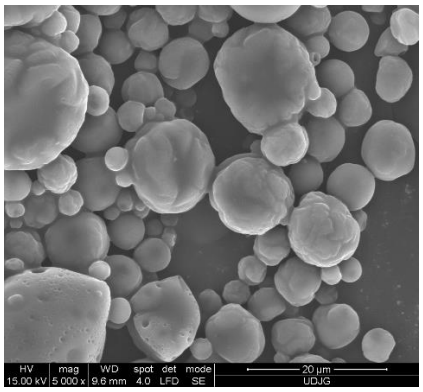
3.1. Preparation and characterization of VD3-GA/Ch microparticles

The spray drying method is one of the most used methods for encapsulating bioactive compounds such as aroma compounds, natural food colorants, fish oils, antioxidants, essential oils and vitamins [31]. The most important factors that influence the quality of the microparticles obtained by spray drying method (encapsulation efficiency, particles size, surface and the release process) are the nature of the encapsulating material; stability of emulsions; the viscosity of encapsulating material solutions; solid substance content; the parameters of the drying process (gas flow; feed rate; the temperature inside and outside the drying chamber) [4, 31]. Considering these aspects, studied in previous our work [3] in the first step of the process a stable O/W emulsion loaded with VD3 was obtained by the sonication method.

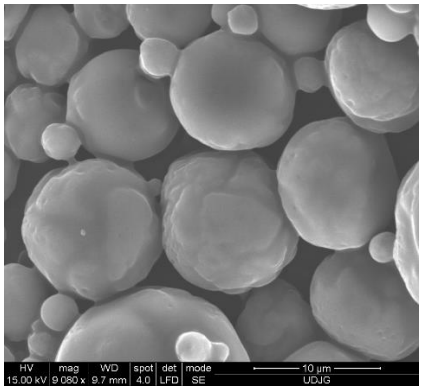
Linseed oil rich in polyunsaturated fatty acids was used as the oily phase. After the spray drying process of O/W emulsions, a fine white powder was obtained.

The encapsulation yield was 65.6%±3.6%, according to other researchers [21, 22]. The low value of the encapsulation yield is due to the adhesion of the powder on the inner surface of the drying chamber due to the chitosan, which has important adhesive properties. The moisture content and encapsulation efficiency of the VD3-GA/Ch microparticles were 3.89±0.16% and 89.78±3.88%, respectively. Other authors reported similar results [32, 33, 34].

The average diameter (d_{43}) of the VD3-microparticles was 12.64±1.14 μm and the particle size distribution showed a monomodal curve, with a SPAN factor of 0.631. SEM images show that the microparticles are spherical with a brain-like surface (Figure 1). This surface structure is specific to chitosan particles reported by other authors [21, 35].



(a)



(b)

Figure 1. SEM images of VD3-GA/Ch microparticles at 5000X (a) and 9000X (b)

3.2. Preparation of pear juice fortified with VD3 encapsulated in GA/Ch microparticles

The pears (*Pyrus bretschneideri Rehd.*) were washed, cleaned, cut with a knife into four parts, cold-pressed and then filtered to obtain a clear juice without pulp and sediment. According to the legislation regarding the tolerable upper intake levels for vitamin D, 50µg/day for 1-3 years and 100µg (4000 IU)/day for 9 years and older [36], to the fortification of pear juice an amount of 0.002 g (VD3-GA/Ch microparticles)/100ml pear juice was used.

The following samples were prepared: *sonicated pear juice unfortified (P1)*; *pear juice sonicated before fortification with VD3-GA/Ch microparticles (P2); pear juice sonicated after fortification with VD3-GA/Ch microparticles (P3).* The control sample (M) was pear juice unfortified and without ultrasound process. The ultrasound process was applied for the preservation of the pear juice [37].

The samples were sealed into sterilized glass bottles and kept at 4ºC. All the determinations were made in triplicate on the first and seventh day of storage.

3.3. Physico-chemical characterization of pear juice fortified with VD3-AG/Ch microparticles

The ripening degree of pears is determined by the total soluble solids (ºBrix), *p*H and titrable acidity. These parameters are influenced by physical and chemical factors, such as fruit origin, plant species and ripeness [38]. Due to the high sugar content, the juice is characterized by a sweet taste. After the ultrasound process no significantly, different values were observed on the total soluble solids (ºBrix, $p>0.05$), *p*H and titrable acidity (Table 1). These results are according to other previous researches [11, 37]. The increased values of titrable acidity for the samples P1, P2 and P3 compared with the control (M) on the first and seventh day of storage are due to the release of citric acids and other phenolic compounds during the ultrasound process [39].

Table 1. Total soluble solids (ºBrix), pH and titrable acidity in pear juice fortified with VD3-loaded GA/Ch microparticles

Sample	ºBrix		*p*H		Titrable acidity (%)	
	1 (day)	7 (days)	1 (day)	7 (days)	1 (day)	7 (days)
M	11.77±0.35	11.17±0.34	4.11±0.01	4.01±0.01	0.21±0.01	0.25±0.01
(P1)	12.03±0.15	11.33±0.35	4.45±0.03	4.25±0.03	0.36±0.01	0.44±0.01
(P2)	11.95±0.25	11.45±0.15	4.23±0.06	4.13±0.02	0.38±0.02	0.41±0.02
(P3)	12.13±0.55	12.03±0.50	4.25±0.02	4.15±0.02	0.31±0.01	0.42±0.01

P1: sonicated pear juice unfortified
P2: pear juice sonicated before fortification
P3: pear juice sonicated after fortification
M: control sample (pear juice)

Table 2. Content of vitamin C and vitamin D3 of pear juice fortified with VD3-loaded GA/Ch microparticles

Samples	Vitamin C (mg AA/100 ml)		Vitamin D3 (µg/100ml)	
	1 (day)	7 (days)	1 (day)	7 (days)
M	3.75±0.05	3.70±0.05	ND	ND
(P1)	3.85±0.02	3.71±0.02	ND	ND
(P2)	3.91±0.02	3.84±0.02	1±0.09	1.1±0.06
(P3)	3.98±0.04	3.85±0.02	1.3±0.07	1.5±0.04

ND: unidentified
P1: sonicated pear juice unfortified
P2: pear juice sonicated before fortification
P3: pear juice sonicated after fortification
M: control sample (pear juice)

The phytochemical characterization of the fortified and ultrasound pear juice showed a high content of phenols (0.187 mg GAE/mL), flavonoids (0.057 mg CE/mL) and high antioxidant activity (0.92 mMol Trolox/mL). There were no differences in the ultrasound samples (Table 3). These results are in agreement with other previous research [11, 41].

During storage, a decrease in total phenol content of over 4% was observed in all ultrasound samples. Flavonoids compounds were stable during storage at 4ºC for seven days. Antioxidant activity in the control sample (M) decreased by 6% while in the P1 sample increased by 5% due to the ultrasound process that broke the plant membrane wall and released a large quantity of compounds with high antioxidant activity.

This result is in agreement with that reported by Ozyurt *et al.* (2019) [44] in apple juice. The P2 and P3 samples showed stability due to the stability of the flavonoid compounds which are responsible for the antioxidant activity.

3.4. Microbiological characterization of pear juice fortified with VD3-AG/Ch microparticles

The microbiological results showed that the application of the ultrasonic process on the natural pear juice fortified with vitamin D3 encapsulated in polymeric microparticles reduced the total number of mesophilic aerobic bacteria from 2.5x10 in the control sample to 1.6x10, 1.5x10 and 1.6x10 respectively in the other samples (P1. P2, P3). The results are in agreement with other previous research [11] and are presented in Table 4.

Table 3. Total phenols, flavonoids and antioxidant activity of pear juice fortified with VD3-loaded GA/Ch microparticles

Samples	Total phenolics (mg GAE/ml)		Total flavonoids (mg CE/ml)		Antioxidant activity (mM Trolox/ml)	
	1 (day)	7 (days)	1 (day)	7 (days)	1 (day)	7 (days)
M	0.177±0.003	0.180±0.003	0.052±0.001	0.050±0.001	0.920±0.025	0.870±0.022
(P1)	0.183±0.005	0.172±0.002	0.056±0.002	0.052±0.001	0.862±0.017	0.904±0.036
(P2)	0.187±0.002	0.174±0.009	0.057±0.001	0.053±0.005	0.896±0.028	0.908±0.018
(P3)	0.181±0.001	0.177±0.006	0.054±0.003	0.059±0.004	0.907±0.019	0.913±0.042

P1: sonicated pear juice unfortified
P2: pear juice sonicated before fortification
P3: pear juice sonicated after fortification
M: control sample (pear juice)

Table 4. Microbiological analysis of pear juice fortified with VD3-loaded GA/Ch microparticles

Type of analysis	Samples							
	(M)		(P1)		(P2)		(P3)	
	1 day	7 days	1 day	7 days	1 day	7 days	1 day	7 days
Mesophilic aerobic bacteria (CFU/ml, x10)	2.5	2.9	1.6	2.2	1.5	2.1	1.6	1.8
Enterobacteriaceae (CFU/ml)	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Yeasts and moulds (CFU/ml)	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1

P1: sonicated pear juice unfortified
P2: pear juice sonicated before fortification
P3: pear juice sonicated after fortification
M: control sample (pear juice)

4. Conclusions

In this study, the fortification of pear juice with VD3 encapsulated in polymeric microparticles prepared by the spray drying method was investigated. In particular, the VD3-loaded microparticles were prepared using as encapsulating material a mixture of gum arabic and chitosan (GA: Ch 9: 1w/w). Vitamin D3, solubilized in linseed oil, was mixed with the aqueous phase containing the polymers and the emulsifier. By spray drying of the O / W emulsion, a white powder was obtained, which contained microparticles with an average size of 12.64±1.14 and a low polydispersity, confirmed by the low value of SPAN factor (0.631). Several physicochemical analyzes of fortified and ultrasound pear juice with VD3-loaded microparticles, such as total soluble solids (°Brix), pH, titrable acidity, vitamin C content, vitamin D3 content, total phenolic content, total flavonoids content, antioxidant activity, and microbiological assays, have been performed. It has also been shown that by ultrasound, the content of bacteria decreases and the content of vitamin D3 increases due to its release from microparticles. The obtained results show that by fortification and ultrasound process of pear juice with vitamin D3 incorporated in polymeric microparticles, a stable beverage was obtained, which retains its physicochemical characteristics for more than seven days. Polymeric microparticles are innovative vitamin delivery systems that can be used to make food functional and beverages.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

Acknowledgement

This work was supported by an internal research grant of "Dunarea de Jos" University of Galati, Romania, contract number GI -02/01.03.2018.

This work was supported by the project "EXPERT", financed by the Romanian Ministry of Research and Innovation, Contract no. 14PFE/17.10.2018.

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