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Micro Encapsulation of Bioactive Compounds through Co Crystallization

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ABSTRACT

Micro encapsulation through co-crystallization technique is allowed to obtain different cocrystallized products with a high concentration of the bioactive compound. The resulting powders are free flowing and stable even at high humidity conditions. Co-crystallized product properties like flowability, solubility, density and size distribution mainly depended on the sucrose matrix. Whereas water activity and hygroscopicity varied with the different active component added. Co-crystallization notably reduced the hygroscopic characteristics of bioactive powders without affecting their high solubility. The co-crystallization process resulted in a good alternative to preserve and handle these materials for further applications in food products. Co-crystallization of sucrose and the pre emulsified oleoresins, essential oils and flavouring agents with gum acacia can be used to obtain flavoured sucrose cubes.

Key words: *Co-crystallization, Encapsulation, Bioactives, Physical properties*

INTRODUCTION

Encapsulation technologies become an actual choice applied to preserve and/or protect numerous ingredients from adverse environmental conditions (light, moisture, and oxygen) and to prevent undesirable interactions with the carrier food matrix[17,22]. In the food industry, the microencapsulation process can be applied for a variety of reasons, which have been summarized by Desai and Park[15] as follows: (i) protection of the core material from degradation by reducing its reactivity to its outside environment; (ii) reduction of the evaporation or transfer rate of the core material to the outside environment; (iii) modification of the physical characteristics of the original material to allow easier handling; (iv) tailoring the release of the core material slowly over time, or at a particular time; (v) to mask an unwanted flavor or taste of the core material; (vi) dilution of the core material when only small amounts are required, while achieving uniform dispersion in the host material; (vii) to help separate the components of the mixture that would otherwise react with one another.

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Food ingredients of acidulants, flavoring agents, sweeteners, colorants, lipids, bioactive compounds, vitamins and minerals, enzymes and microorganisms, are encapsulated using different technologies[15]. Fang and Bhandari[16] and Munin and Edwards-Lévy[21] reviewed the different microencapsulation techniques employed for the encapsulation of bio-actives such as spray drying, coacervation, liposome entrapment, inclusion complexation, co-crystallization, nanoencapsulation, freeze drying and emulsion. Spray drying is the most widely applied process mainly for the preparation of dry, stable food additives and flavours[15]. Among various microencapsulation processes, co-crystallization is a relatively recent development[10]. Co-crystallization is a process whereby a second (active) ingredient is embedded inside the conglomerate of crystals. Sucrose is used as a primary ingredient. Co-crystallization offers an economic and flexible alternative for the incorporation of active compounds into powder foods.

Micro encapsulation of bio-active compounds by Co-crystallization

Co-crystallization is an encapsulation process in which the crystalline structure of sucrose is modified from a perfect to an irregular agglomerated crystal, to provide a porous matrix in which a second active ingredient can be incorporated[11]. Spontaneous crystallization of supersaturated sucrose syrup is achieved at high temperature (above 120°C) and low moisture (95-97°Brix). If a second ingredient is added at the same time, the spontaneous crystallization results in the incorporation of the second ingredient into the void spaces inside the agglomerates of the microsized crystals (Fig. 1), with a size less than 30 mm[6]. The main advantages of cocrystallization are improved solubility, wettability, homogeneity, dispersibility, hydration, anticaking, stability and flowability of the encapsulated materials[9]. Other advantages are that the core materials in a liquid form can be converted to a dry powdered form without additional drying, and the products offer direct tableting characteristics because of their agglomerated structure, and thus offer significant advantages to the candy and pharmaceutical industries[15].

Very few research works have been published on co-crystallization, although a number of patents have been reported[2,11]. described numerous products that can be encapsulated by co-crystallizing with sucrose such as: fruit juices, essential oils, flavours, peanut butter, chocolate, brown sugar etc[4]. Encapsulated orange peel oil using a co-crystallization process and conducted a storage test on the resultant product[1]. prepared a blend of commercial sucrose (50g) and yerba mate extract (10 mL) was heated to 132°C on a hot plate and stirred with a vertical agitator. When a slight turbidity was detected in the syrup, indicating the beginning of crystallization process, the mix was removed from the heat, maintaining the agitation. Then, co-crystallized products were dried in a convection oven at 40°C for 15 h, milled and transferred to polyethylene bags for storage in desiccators with silica gel until use. Deladino, Anbinder, Navarro, and Martino[12] reported on the encapsulation of yerba mate (*I. paraguariensis*) extract containing caffeoyl derivatives and flavonoids, by cocrystallization in a supersaturated sucrose solution. The co-crystallized product had a typically cluster-like agglomerate structure with void spaces and a sucrose crystal size varying between 2 and 30 mm. An extra layer of a network with neat edges covered the crystals. The microstructure was further confirmed by differential scanning calorimetry, X-ray diffraction and scanning electron microscopy[14]. The cocrystallization of yerba mate extract changed it from a cohesive material to be a non-cohesive product, and notably reduced its hygroscopic characteristics without affecting its high solubility; this demonstrated that cocrystallization is a good alternative for the preservation and handling of yerba mate extract for further application in food products. There have been very few reports of the application of the cocrystallization process. Antioxidant aqueous extracts of yerba mate were entrapped into a sucrose matrix by co-crystallization. The

products were characterized in terms of their morphology, entrapment yield, loading capacity, water activity, moisture content, antioxidant activity, thermal behavior, solubility and hygroscopicity. It was found that the co-crystallization process led to high entrapment yield and maintained the antioxidant activity of the yerba mate extract towards DPPH radical. Also, the co-crystallized powders showed values of water activity, moisture content, hygroscopicity and flowability indicative of high stability and good handling properties. The total polyphenols content of the co-crystallized products remained almost constant along storage at 75% RH and 20°C, however, fluctuations in their DPPH radical scavenging activity were observed. B.R. Sardar, R.S. Singhal were encapsulated the Cardamom oleoresin with emulsified gum acacia by co-crystallization in a supersaturated sucrose solution to prepare flavoured sucrose cubes. The co-crystals with and without cardamom oleoresin were characterized at 25°C and 33%, 63% and 93% relative humidity for hygroscopicity and crystallinity vis-à-vis pure crystal sucrose. Co-crystallized sucrose cubes showed lower hygroscopicity at 93% relative humidity, a longer dissolution time, and a decreased crystallinity as compared to crystal sucrose. The active components of cardamom oleoresin such as 1,8-cineole (30.23%) and a-terpinyl acetate (46.42%) in cardamom oleoresin was quantified by gas chromatography. The encapsulation efficiency of 1,8-cineole and a-terpinyl acetate in lab-made co-crystallized sucrose cubes was approximately 35.23% and 67.18%, respectively. This approach could contribute to value addition of cardamom oleoresin for flavoured tea and also have potential applications in traditional Indian sweets. Deladino *et al*.[14]. were prepared a blend of mineral with yerba mate extract by crystallization of a sucrose syrup of 71.5% w/w, 10 g of total supersaturated solution were obtained by heating 50 g of sucrose in water in a metallic vessel with continuous stirring. When a slight turbidity was detected in the syrup, indicating the beginning of crystallization process from the solution, the second compound was rapidly added to the syrup[7]. Mineral salts (5 g magnesium sulphate and 2 g calcium lactate per 100 g sucrose) were incorporated in powder form or in solution to evaluate the effect of their physical state on the final product, whereas antioxidant extract (0.7 g/100 g sucrose) was added in powder form. At this moment the mixture was placed over a cold surface, with continuous stirring until a solid product was obtained. Crystallized product obtained from 71.5% sucrose syrup without any other component was taken as control. Co-crystallized products were transferred to a glass vessel and stored for 24 h in a desiccator. Deladino *et al*.[14], were made a co-crystallized product of minerals with sucrose syrup matrix was prepared according to Deladino *et al*.[14]. The sucrose syrup was heated in a metallic vessel with constant agitation. Once turbidity was observed, indicating the crystallization beginning, the active compound was quickly added: 5 g of magnesium sulphate ($MgSO_47H_2O$), 2 g of calcium lactate ($CaC_6H_{10}O_65H_2O$) or 0.7 g of lyophilized antioxidants extract (*Ilex paraguariensis*), in all cases in a 100 g sucrose basis. After active compound addition, heating was stopped and agitation continued until a solid product was obtained. The heating was monitored with thermocouples; maximum temperature reached in the process was 140°C. The final product was maintained 24 h in a dissecator. Then it was dried in a vacuum oven at 60°C, grounded and sieved. Beristain *et al*.[9], Microencapsulated Orange peel oil by co-crystallization. Encapsulation capacity of sucrose syrups was found to be greater than 90% for a range of 100 to 250 g oil/kg of sugar. Surface oil, a measurement of encapsulation efficiency, varied from 3350 to 8880 mg oil/kg solids. Moisture content of the crystals was lower than 10 g/kg and bulk density was greater than 670 kg/m3for all the co-crystallizates prepared. Sensory evaluation showed that all of the panelists were able to detect oxidized flavours in oils without

antioxidant added after storage at 35 °C for 1 d. When BHA was added to the oil prior to co-crystallization, no signs of oxidized flavours were detected after 2 months of storage at ambient temperature. Bhandarif *et al.*[8], Co-crystallized Honey was with a sucrose syrup at 128°C using a sucrose :honey proportion of 90:10, 85:15 and 80:20. The first two proportions produced granular co-crystals, whereas the ratio of 80:20 produced a semi-solid product. The granules were relatively free flowing with an angle of repose 38.5–39.5°. Perkkalainen *et al.*[23], co crystallized mixtures of sugar alcohols xylitol, D-sorbitol and D-mannitol by grinding the solid starting materials together for 20 min. possible co-crystals were identified by DSC and X-ray powder diffraction. The phase diagrams for the systems xylitol-D-sorbitol, xylitol-D-mannitol and D-sorbitol-D-mannitol were created using the peak values from the DSC measurements as melting points. The phase diagram for xylitol and D-sorbitol showed that co-crystallization between these two components may be achieved by grinding them together. The powder diffraction pattern for a mixture of 0.50 mole fraction of xylitol and 0.50 mole fraction of D-sorbitol showed that the product consisted of both starting reagents and a new co-crystallization product. The melting point of the co-crystallization product was 75–77°C measured from the peak value of DSC measurements. María Clara Zamora and Jorge Chirife[20] As most honeys are supersaturated solutions of glucose, this sugar may crystallize spontaneously at room temperature in the form of glucose monohydrate. Crystallization of honey lowers the glucose concentration in the liquid phase and thus increases the water activity (aw) which sometimes can allow naturally occurring yeasts cells to multiply, causing fermentation of the honey. It is the purpose of present work to measure the water activity of 49 samples of crystallized honeys from Argentina, as well as the shift in water activity (aw) when the samples were re-dissolved upon heating. It was found that Δaw for most samples studied was in the range 0.03–0.04 aw. Studies with sugar model systems resembling honey confirmed that the observed change in water activity quantitatively corresponded to that caused by glucose crystallization.

Characterisation of Co-crystallized products

The co-crystallized products are characterized by the following methods

Solubility

Solubility is determined by blending 1 g of co-crystallized powder with 10 mL of distilled water at ambient temperature with continuous stirring. Aliquots are removed at different times and the dissolved sucrose mass in the solution was determined using a refractometer.

Water activity, dissolution time and hygroscopicity

Water activity (aw) of crystal sucrose and co-crystallized products is determined using Aqua Lab Series 3 TE (USA) equipment. Dissolution time is determined by manually adding crystal sucrose and co-crystallized sucrose cubes (10 g) to 100 ml distilled water with continuous stirring at 400 rpm and 25°C (Beristain et al., 1994). Hygroscopicity (HG) is calculated from the equation given by Jaya and Das[18], as water gained by the sample on a dry basis as follows:

$$HG\ \% = b + H/a\text{-}H \times 100$$

where b (g) is the weight increase, a (g) is the initial sample weight and H is the initial water content of the sucrose cubes (g). Initial water content (H) was determined by drying the ground co-crystallized sucrose cubes samples in a vacuum oven at 60°C, until constant weight.

Flowability tests

Flowability of the co-crystallized powders is determined by both, dynamic angle of repose and Hausner ratio (H). The angle of repose is determined with a rotating cylindrical chamber, which is tilted gradually until slipping occurred and the angle measured (Solids handling study bench, CEN, Armfield, United Kingdom). The value of H is calculated by the ratio of the tap bulk density to the loose bulk density. The loose bulk density is determined by pouring a known mass of co-crystallized product delivered

freely by gravity into a measuring cylinder, and it is calculated by dividing the mass by the bulk volume. The tap bulk density is calculated from the weight of powder contained in the cylinder after being hand tapped 100 times at roughly 60 taps/min. All measurements were performed at least in triplicates.

Sorption isotherms

Sorption isotherms are obtained equilibrating 10 g of sample in glass petridish at 25°C and different relative humidities (RH) within the range 33–93%. Saturated salt solutions used are magnesium chloride (RH 33%), sodium nitrite (RH 63%), and potassium nitrite (RH 93%). Sorption isotherms of pure crystal sucrose, co-crystallized sucrose cubes with and without carrier materials emulsified with core materials are determined.

Scanning electron microscopy (SEM)

Samples are analyzed in a scanning electron microscope (SEM, Jeol, JSM-6380LA). Co-crystallites are attached to SEM stubs using a two-sided adhesive tape, and then coated with a layer of gold (40–50 nm) and examined using an acceleration voltage of 10 kV and 15 kV for pure crystal sucrose and co-crystallized sucrose samples, respectively.

Differential scanning calorimetry (DSC)

Thermal behavior is studied by using a differential scanning calorimeter (DSC–60), thermal analyzer (TA–60WS), flow control (FC–60A) (Shimadzu, Japan). Crystal sugar and co-crystallized sucrose samples are cooled to 40°C below the expected glass transition temperature (Tg) and scanned in hermetically sealed pans under a nitrogen flow of 10 ml/min used to purge the sample head and glove box. Heat flow and temperature calibration is performed with indium. The melting temperatures (Tm) and enthalpy (DH) of melting of pure crystal sucrose, co-crystallized sucrose cubes with and without core material are determined at a scan rate of 10°C/min with heating scan up to 250°C using 4 mg of sample in hermetically sealed aluminum pan.

X-ray diffraction (XRD)

The equipment used is an X'Pert PRO (Holland) at 40 kV with radiation of wavelength of 40 mÅ. Samples are scanned with 2h between 5 and 60 degree. Co-crystallized products are dried at 60°C in a vacuum oven before the assay. X-ray diffraction of pure crystal sucrose and co-crystallized sucrose cubes with and without core materials are also determined.

CONCLUSION

Co-crystallization technique is allowed to obtain different cocrystallized products with a high concentration of the active compound. The resulting powders are free flowing and stable even at high humidity conditions. Co-crystallized product properties like flowability, solubility, density and size distribution mainly depended on the sucrose matrix. Whereas water activity and hygroscopicity varied with the different active component added. Co-crystallization notably reduced the hygroscopic characteristics of bioactive powders without affecting their high solubility. The co-crystallization process resulted in a good alternative to preserve and handle these materials for further applications in food products. Co-crystallization of sucrose and the pre emulsified oleoresins, essential oils and flavouring agents with gum acacia can be used to obtain flavoured sucrose cubes. The co-crystals can be used directly. It opens up newer vistas for use of sucrose and flavouring components in various food applications especially in traditional Indian sweets. Further research is being pursued to determine the crystallization of individual principal sugars in a mixture of sucrose and desired core material during the co-crystallization process.

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