



Microstructure of minerals and yerba mate extract co-crystallized with sucrose

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ABSTRACT

Co-crystallization with sucrose is an interesting alternative for handling and preservation of different active compounds used in the food industry. Sucrose products containing different active compounds (calcium lactate, magnesium sulphate and yerba mate extract) were obtained by co-crystallization. The microstructure of these products and raw materials was analyzed by Differential Scanning Calorimetry (DSC), X-rays Diffraction (XRD) and Scanning Electron Microscopy (SEM). The co-crystallized products showed the basic crystalline structure of the treated sucrose without active compound. Mineral distribution in the co-crystallized products was uniform as seen by Energy Dispersive X-rays (EDX). DSC and X-rays analysis showed that the salts were partially dehydrated during the process, yielding products with a crystalline sucrose matrix and, both, anhydrous and hydrated forms of the salts. This characterization helped understanding macroscopic behaviour of the products.

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1. Introduction

Fortification has become a common technique in the food industry, however among others, aspects related to handling during processing, storage or taste of the products should be improved. Flowability, hygroscopicity and wettability of mineral salts are of important concern. Calcium lactate has some advantages like solubility and bioavailability, however, its poor flowability is a drawback for manufacturers (Gerstner, 2003; Deladino et al., 2007). The bitter taste associated with calcium chloride and magnesium salts has been worked out. Lawless et al. (2003) overcame taste problems of calcium ion by combining it with large organic ions such as lactate, gluconate or glycerophosphate. Magnesium sulphate is employed as gustatory stimuli for bitterness (Hodson and Linden, 2006). Nowadays, other interesting compounds used in food fortification are antioxidants extracted from natural sources. However, some antioxidant extracts, like lyophilized yerba mate powder, show high hygroscopic behaviour and low flowability. Deladino et al. (2007), in a previous work, applying an encapsulation process with sucrose provided an alternative to solve these unattractive characteristics of yerba mate extracts and calcium and magnesium salts.

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 where a second active ingredient can be incorporated (Chen et al., 1988). This technique allows incorporating hard-to-handle active compounds in a free-flowing carrier, protecting them against oxidation or other undesired chemical and physical changes (Awad and Chen, 1993; LaBell, 1991). Since the late eighties, different authors have obtained powder products through co-crystallization technique (Beristain et al., 1994, 1996; Bhandari et al., 1998; Bhandari and Hartel, 2002; Vázquez and Beristain, 1998). Hygroscopicity, flowability and water sorption isotherms of inorganic salts and organic extracts co-crystallizates were determined in a previous study (Deladino et al., 2007). The macroscopic characteristics highly depend on the physical structure of the powder products. Thus, further microstructure characterization was faced using Differential Scanning Calorimetry (DSC), X-rays Diffraction (XRD) and Energy Dispersive X-rays (EDX) to highlight this point.

DSC is probably the most common technique in the determination of phase transitions in organic, inorganic, polymeric and also food materials. Zeng et al. (2001) characterized the presence of amorphous structures in samples of co-lyophilized sucrose with polyvinylpyrrolidone through DSC. Glass transition is evidenced by a change in specific heat, which is followed by a crystallization exotherm happening at a higher temperature. This

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transition revealed the amorphous form of sucrose products since crystalline sucrose only has a melting endotherm at 180 °C. Hurтта et al. (2004) studied the melting behaviour of D-sucrose, D-glucose and D-fructose, concluding that melting point determination by DSC is a sensitive method for the characterization of the crystal quality. However, the melting of sugars is a multiphase phenomenon and the results could be affected by both the determination method and the sample history. The crystalline structure of carbohydrates can be also elucidated through X-ray diffraction studies. Bhandari and Hartel (2002) used this technique to identify changes in the crystalline structure of sucrose and other added sugars during storage of co-crystallized products.

The objective of the present work was to characterize co-crystallized products through DSC, X-rays and SEM-EDX techniques to determine the microstructure properties of the co-crystallized agglomerates that could help in understanding macroscopic behaviour of these powder materials.

2. Materials and methods

2.1. Sample preparation

Sucrose syrup of 71.5% w/w was prepared according to Deladino et al. (2007). 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 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 2 g of calcium lactate ($\text{CaC}_6\text{H}_{10}\text{O}_6 \cdot 5\text{H}_2\text{O}$) 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 desiccator. Then it was dried in a vacuum oven at 60 °C, grounded and sieved. The maximum particle size was 1 mm in diameter. A co-crystallized sample of sucrose without active compound was taken as a control (treated sucrose). Final water content was determined drying the samples until constant weight.

2.2. Product characterization

Samples were analyzed in a scanning electron microscope Jeol JSM-6360LV (Japan). Co-crystallized samples were attached to SEM stubs using a two sided adhesive tape, then they were coated with a layer of gold (40–50 nm) and examined using an acceleration voltage of 10 kV. The samples were subjected to energy dispersive X-rays (EDX) analysis in order to find out the distribution of elements (Econ 4, EDX, USA).

Thermal behaviour was studied by DSC technique using a calorimeter (Polymer Laboratories, United Kingdom), with a heating rate of 10 °C/min, from 50 to 250 °C. For each analysis, 3 mg of dried sample were weighed precisely into a hermetically sealed aluminium pan.

Identification of crystalline structures in co-crystallized products was achieved by powder XRD, the equipment used was an X'Pert PRO (Holland) at 40 kV with radiation of wavelength of 40 mÅ. Samples were scanned with 2θ between 5° and 60°. Mineral salts were dried at 60 °C in a vacuum oven before the assay. Peaks of diffractograms were compared with a database (1999 JCPDS-International Centre for Diffraction Data. PCPDFWIN v. 2.02).

3. Results and discussion

Active compounds (antioxidants extract of yerba mate, calcium lactate and magnesium sulphate) were co-crystallized in a sucrose matrix in order to improve their poor characteristics of flowability and hygroscopicity (Deladino et al., 2007). The moisture contents of the dried co-crystallized products were 0.079% w/w for treated sucrose, 0.055% w/w for yerba mate extract, 0.127% w/w for calcium lactate and 0.480% w/w for magnesium sulphate.

SEM micrographs show that treated sugar matrix is agglomerated (Fig. 1a), the formation of clusters of crystals left irregular cavities within the agglomerates. Yerba mate product (Fig. 1b) shows an extra layer covering the crystals, losing the neat edges seen in treated sucrose. The same type of agglomerates, with holes or void spaces, was observed in mineral products showing crystals of different size (Fig. 1c and d). SEM-EDX micrographs indicated a uniform distribution of calcium and magnesium ions on each product surfaces (Fig. 1e and f). SEM micrograph with higher magnification of co-crystallized MgSO_4 (Fig. 1h) shows a brighter surface and well defined cubic crystals that could be attributed to this salt as compared to treated sucrose (Fig. 1a). On the other hand, calcium lactate co-crystallized photographs show a fibrous or needle-like surface (Fig. 1g). Sakata et al. (2005) also described as needle-like the morphology of pentahydrate calcium lactate when studying different salt hydrates during dehydration process.

Melting endotherms obtained by DSC analysis (Fig. 2) evidenced that sucrose is in crystalline state after co-crystallization process. Thermograms of commercial sucrose and treated sucrose without active compound, presented two transitions with peak temperature values of around 190 and 235 °C (Table 1, Fig. 2a and b). The first endothermic peak corresponded to melting of pure sucrose (Bhandari and Hartel, 2002; Hurтта et al., 2004; Roos, 1995; Zeng et al., 2001). The peak at around 235 °C could be attributed to the degradation of sucrose; the heat of fusion of this peak was not constant, since during decomposition, different compounds could be formed each time. According to Roos (1995), caramelization occurs especially in sugars that have melting temperatures above 150 °C.

When analyzing co-crystallized mineral salt products, differences with respect to the raw salts were found (Fig. 2a and b). In the case of raw calcium lactate, it was found a melting peak at 93.9 °C and a second peak at 192.6 °C (Fig. 2a, Table 1). Similarly, Sakata et al. (2005) found an endothermic peak at 95.9 °C for calcium lactate, which was attributed to the transition from the pentahydrate to the anhydrous form by thermogravimetric analysis. As calcium lactate used in this study was of food grade, the peak at 192.6 °C could be due to impurities, since literature data does not provide evidence of other transitions for this salt. In co-crystallized calcium lactate, the peak at 93.9 °C was not observed, which suggested that the salt might have been dehydrated during co-crystallization, since a temperature around 140 °C was reached in the process. A peak at 190 °C was observed and it was attributed to the typical sucrose melting (Fig. 2b, Table 1). The slight broadening of this peak could be due to the contribution of calcium lactate peak at 192.6 °C. An exothermic peak (not quantified because of experimental considerations) was observed between 216–226 °C. This peak was associated to the possible interaction between salt impurities and sucrose and/or sucrose caramelization products. Besides, the endothermic peak at 231.5 °C attributable to sucrose matrix was overlapped with the above mentioned exothermic peak.

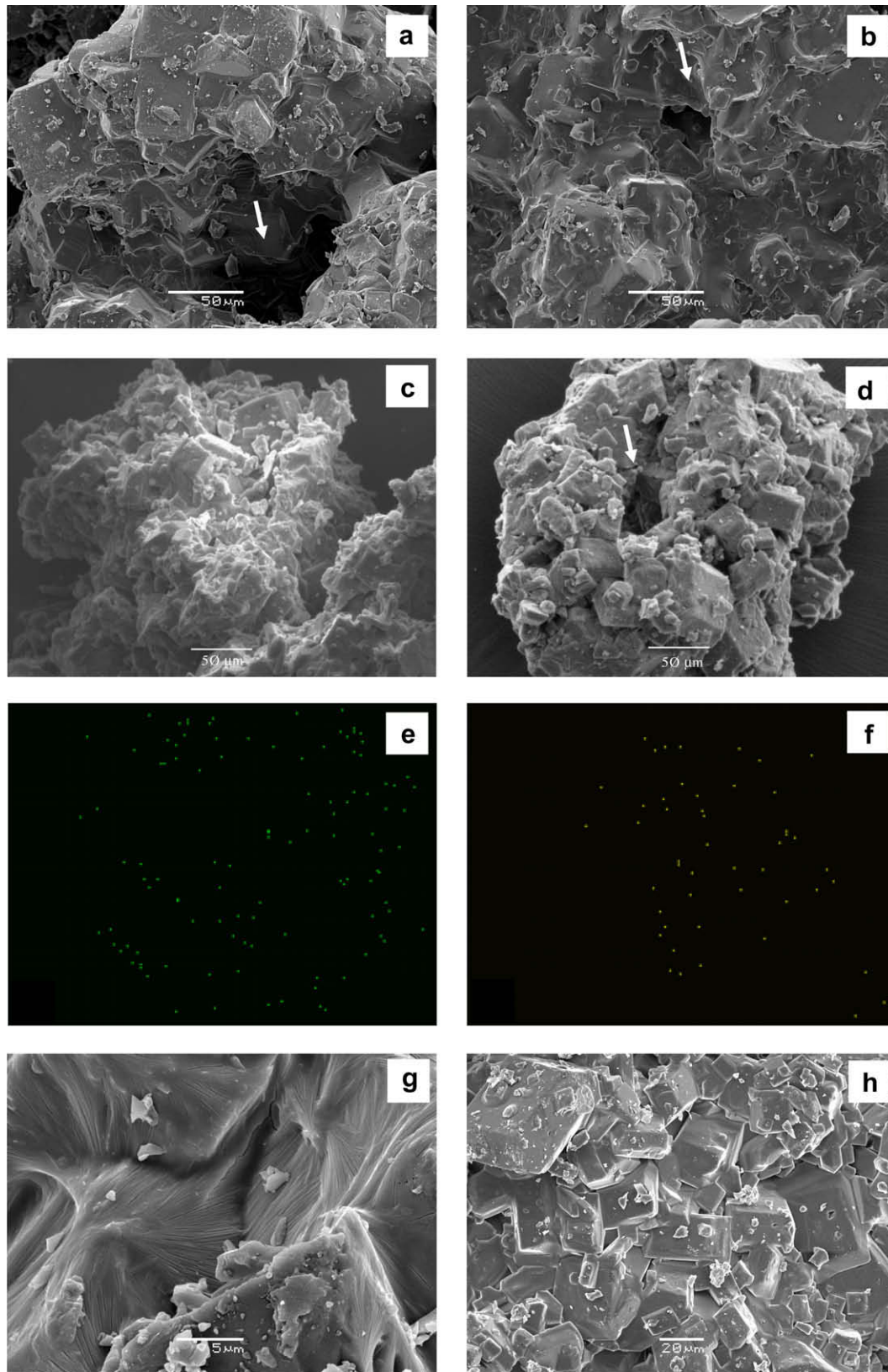


Fig. 1. SEM microphotographs of (a) treated sucrose (b) co-crystallized yerba mate extract; SEM microphotographs and EDX mineral mapping of co-crystallized calcium lactate (c, e and g) and co-crystallized magnesium sulphate (d, f and h). The white arrows indicate the voids or cavities left by the clusters of crystals.

Magnesium sulphate raw salt exhibited a three peak pattern (Fig. 2a). Emons et al. (1990) and Paulik et al. (1981) studied

the dehydration of magnesium sulphate heptahydrate by thermogravimetric and X-rays measurements. These authors determined

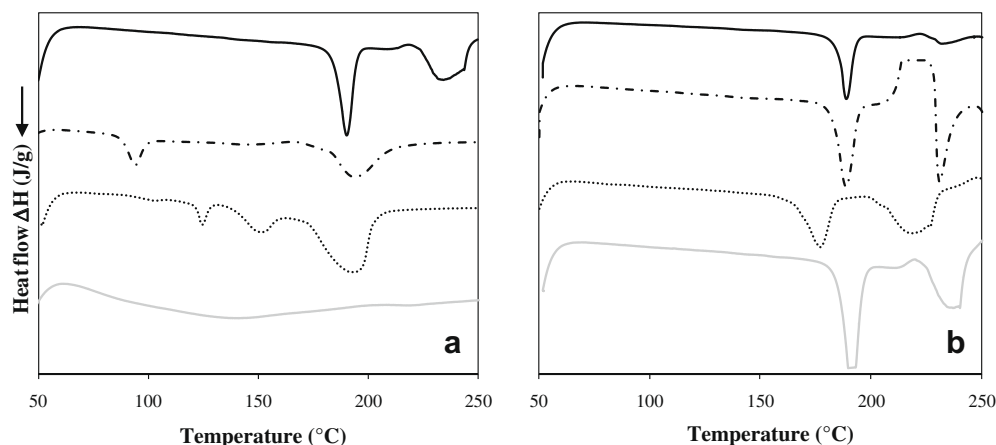


Fig. 2. Thermograms obtained by Differential Scanning Calorimetry of (a) Commercial crystalline sucrose and pure active compounds and (b) Co-crystallized products; — sucrose, - - - calcium lactate, magnesium sulphate and — yerba mate extract.

Table 1

Data values obtained from DSC endotherms for co-crystallized products and their corresponding controls.

Sample	Peak no.	Onset temp. (°C)	Peak temp. (°C)	ΔH (J/g)
Sucrose	1	184.28 ± 0.47	190.22 ± 0.31	99.54 ± 6.45
	2	222.76 ± 0.71	235.68 ± 2.56	103.43 ± 7.93
Co-crystallized sucrose	1	182.58 ± 0.55	187.67 ± 0.01	100.63 ± 0.60
	2	223.88 ± 0.29	235.52 ± 0.43	63.42 ± 2.62
Calcium lactate	1	87.58 ± 0.05	93.88 ± 0.05	30.34 ± 2.00
	2	176.46 ± 3.31	192.60 ± 0.71	94.62 ± 15.12
Co-crystallized calcium lactate	1	185.24 ± 3.09	190.00 ± 1.50	108.82 ± 3.60
	2	138.73 ± 0.40	144.78 ± 11.78	38.46 ± 4.34
Magnesium sulphate	1	120.49 ± 0.54	124.38 ± 0.44	20.19 ± 6.88
	2	170.42 ± 1.18	190.87 ± 0.85	217.66 ± 21.64
	3	170.42 ± 1.18	190.87 ± 0.85	217.66 ± 21.64
Co-crystallized magnesium sulphate	1	167.94 ± 2.03	176.70 ± 0.96	100.75 ± 3.07
	2	203.44 ± 3.05	219.22 ± 1.64	126.68 ± 8.03
Yerba mate extract	1	55.03 ± 7.61	145.50 ± 1.75	638.55 ± 7.14
Co-crystallized yerba mate extract	1	185.35 ± 0.88	188.01 ± 2.06	128.25 ± 31.96

that the decomposition of the trihydrate form of magnesium sulphate was at 115 °C, however a previous overheating to 130 °C was needed for nucleus formation. In the present work, the first peak found at 124.38 °C correlated with these observations (Table 1). The other two peaks found in this work (144.78 and 190.87 °C) were attributed to the transition to the monohydrate, since according to these authors $MgSO_4 \cdot 2H_2O$ released 1 mol H_2O within the range of 150–200 °C. Co-crystallized salt showed the two endothermic peaks corresponding to the co-crystallized sucrose (Fig. 2b), shifted to lower temperatures, probably due to overlapping or co-melting with magnesium sulphate crystals. The missing of peaks corresponding to transitions below 150 °C suggested that magnesium sulphate has lost its water molecules during co-crystallization, due to the high temperatures achieved in this process.

Yerba mate extract shows a broad peak (Fig. 2a, Table 1), that could be attributed to its complex composition. Co-crystallized yerba mate extract shows the characteristic peak of sugar melting (Fig. 2b, Table 1). A second peak with a maximum at 228–232 °C was observed, which was not very repetitive between replications (data not shown). This last peak was assigned to the caramelization of sucrose in the presence of the extract compounds.

Fig. 3a shows the X-rays diffraction pattern obtained for commercial sucrose, the main ingredient of co-crystallized products.

Diffraction patterns of commercial and treated sucrose did not show significant differences in peak positions (Figs. 3a and b). The pattern found by X-rays and the melting peaks of DSC for control and treated sucrose confirms that sucrose was in crystalline state after co-crystallization process. The peaks observed in X-rays spectrum matched with those in the database for standard reference materials (JCPDS, 1999), peaks with the higher relative intensities appeared at 18.85°, 19.62° and 24.79°. Diffractogram of treated sucrose maintained better the relative intensities of database compared to commercial sugar. For example, the intensity of the characteristic peak at 24.79° was higher than that at 25.22° for the treated sucrose as found in the database. However, the opposite relationship is observed for the commercial sugar sample (Figs. 3a and b).

All co-crystallized products (Figs. 3c and 4c and d) have the same X-rays crystalline pattern of treated sucrose (Fig. 3b), probably due to the low content of the active compound compared to the sucrose matrix. Lyophilized yerba mate extract did not contribute with new peaks (Fig. 3c); indicating that the extract did not crystallize and could have been incorporated into the voids left in sucrose agglomerates (Fig. 1b). Diffractogram of raw calcium lactate was characteristic of the amorphous state (Fig. 4a). Sakata et al. (2005) obtained a similar pattern for anhydrous calcium lactate. These authors reported that the pentahydrate calcium salt exhibits a high degree of crystallinity, whereas

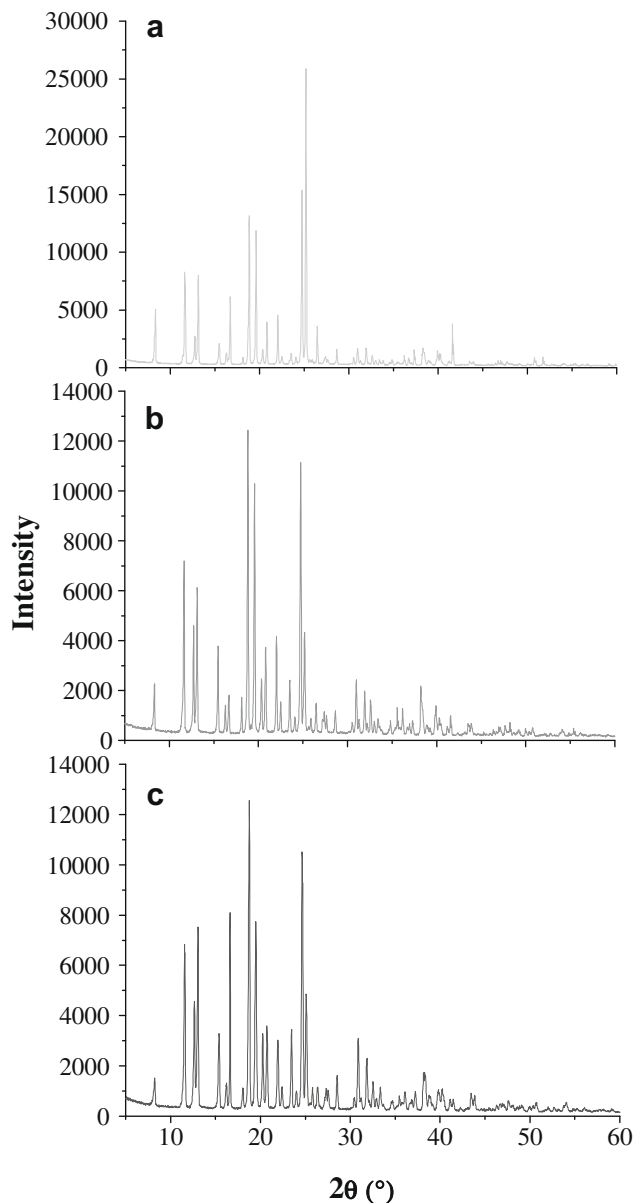


Fig. 3. X-rays diffraction patterns of: (a) commercial sucrose, (b) treated sucrose and (c) co-crystallized yerba mate extract.

the anhydrous salt is amorphous. In the present work, control sample (raw salt) was heated in an oven at 60 °C before the X-rays analysis, thus the loss of the crystalline structure could have been expected (Fig. 4a). The co-crystallized calcium lactate showed crystalline structure (Fig. 4c), with peaks corresponding to treated sucrose (Fig. 3b) and pentahydrate lactate salt. Typical peaks of the pentahydrated form at 7.36°, 8.91°, 22.00° and 24.80° were detected, as reported by Sakata et al. (2005) and standard reference materials (JCPDS, 1999). These results indicated that pentahydrate calcium salt added did not undergo a total dehydration during processing, as was suggested by DSC results. The presence of anhydrous and hydrated forms of the salt would co-exist in the co-crystallized product. Maulny et al. (2005) working on co-crystalline sucrose with honey also found the second ingredient in an amorphous state within the co-crystallized products.

Diffraction peak analysis of raw magnesium sulphate showed a high number of peaks (Fig. 4b). In the present work, drying of control sample at 60 °C led to different salt hydrates. According to the database, peaks at 20.80°, 28.13°, 29.18° and 35.94° corresponded to the tri-hydrated salt, low intensity peaks at 26.17°, 26.60°, 26.91° and 35.05° corresponded to the monohydrate and, finally, a peak at 21.15° was attributed to the hepta-hydrated MgSO_4 (Fig. 4b). The presence of crystals of magnesium sulphate hydrates was also observed by DSC analysis since peaks of hydrates fusion were observed (Fig. 2a). Paulik et al. (1981) found that successive dehydration steps take place during heating of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and characterized different salt hydrates. These authors stated that the heptahydrate decomposes at 115 °C in a mixture containing mono- and di-hydrates states. This temperature is lower to the maximum reached in the present work during co-crystallization process (140 °C). The diffraction pattern of co-crystallized product (Fig. 4d) did not show peaks different from the co-crystallized control matrix (Fig. 3b). Considering that mono-hydrated salt and sucrose crystallize in monoclinic form, probably the mono-hydrated form was present in the co-crystallized product but peaks might have been overlaid. Besides, the possible presence of other hydrates in co-crystallized magnesium salt should be taken into account, based on DSC thermograms where peaks were broadened and shifted to lower temperatures.

As have been discussed before, some structure modifications can be detected by DSC, however these changes could not be observed by X-rays measurements. Thus, performing several techniques is necessary to achieve trusting results as it was demonstrated in previous studies: Bhandari and Hartel (2002) studied the structure modifications of co-crystallized sugar with glucose and fructose during storage; similarly, Maulny et al. (2005) studied the changes of the physical properties of co-crystalline sugar and honey after several months. In both studies, crystallized glucose monohydrate was detected upon storage by DSC. However, in none of the two works X-rays patterns revealed the presence of glucose monohydrate.

Insight into microstructure properties of co-crystallized products allowed understanding flowability and hygroscopicity determined in the previous work (Deladino et al., 2007). In the case of magnesium sulphate, the higher hygroscopicity of the co-crystallized product compared with treated sucrose (12% vs. 2% at 93% of relative humidity, respectively) could be explained by the lower peak temperatures and the broadened transitions observed by DSC measurements.

Repose angle is an index to classify flowing properties of powder materials. According to Peleg (1977) repose angles above 50° or more cause flow problems. Yerba mate extract was so sticky that repose angle could not be determined; its high hygroscopicity strongly affected the flowing properties of this powder. In the mentioned previous work we overcame this problems with the co-crystallization treatment that led to a free-flowing product (repose angle = 39.7°). X-rays analysis in the present work evidenced that the crystalline characteristics of sucrose controlled the flowing properties, since yerba mate extract is contained into the voids of sucrose agglomerates and remains amorphous in the co-crystallized product. Similarly, the low free-flowing characteristics of raw calcium lactate (repose angle = 65.9°) were improved by the co-crystallization process (repose angle = 40.4°) (Deladino et al., 2007). The structure of the raw salt was modified after this process adopting the benefits of crystalline sucrose as determined by DSC and X-rays.

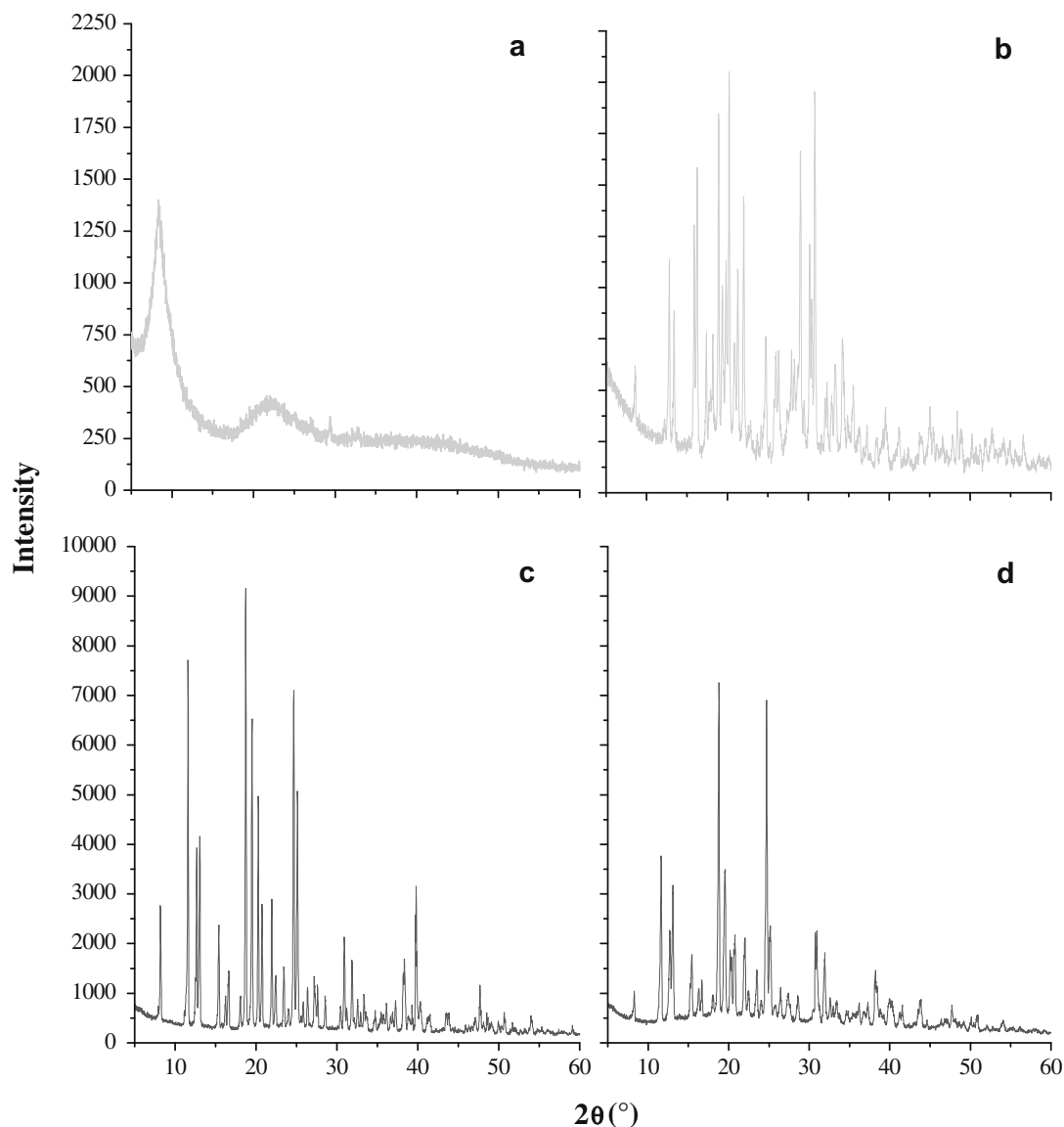


Fig. 4. X-rays diffraction patterns of (a) calcium lactate salt, (b) magnesium sulphate salt, (c) co-crystallized calcium lactate and (d) co-crystallized magnesium sulphate.

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