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Production and properties of casein hydrolysate microencapsulated by spray drying with soybean protein isolate

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ABSTRACT

The aim of this work was to encapsulate casein hydrolysate by spray drying with soybean protein isolate (SPI) as wall material to attenuate the bitter taste of that product. Two treatments were prepared: both with 12 g/100 g solids and containing either two proportions of SPI: hydrolysate (70:30 and 80:20), called M1 and M2, respectively. The samples were evaluated for morphological characteristics (SEM), particle size, hygroscopicity, solubility, hydrophobicity, thermal behavior and bitter taste with a trained sensory panel using a paired-comparison test (non-encapsulated samples *vs.* encapsulated samples). Microcapsules had a continuous wall, many concavities, and no porosity. Treatments M1 and M2 presented average particle sizes of 11.32 and 9.18 μm, respectively. The wall material and/or the microencapsulation raised the hygroscopicity of the hydrolysate since the free hydrolysate had hygroscopicity of 53 g of water/100 g of solids and M1 and M2 had 106.99 and 102.19 g of water/100 g of solids, respectively. However, the hydrophobicity decreases, the absence of a peak in encapsulated hydrolysates, and the results of the panel sensory test considering the encapsulated samples less bitter ($p < 0.05$) than the non-encapsulated, showed that spray drying with SPI was an efficient method for microencapsulation and attenuation of the bitter taste of the casein hydrolysate.

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1. Introduction

The utilization of protein hydrolysates and/or peptides with specific biological activity for nutraceutical food formulation (e.g., athletes, seniors) is of great interest in research as well as for industrial applications. Incorporation of bioactive compounds into food provides a simple way to develop novel functional foods that may have physiological benefits or reduce the risk of diseases (Chen, Remondetto, & Subirade, 2006).

The development of bitterness in the enzymatic hydrolysis due to the release of hydrophobic groups (especially for casein hydrolysates) may limit the use of protein hydrolysates in the food processing industry. In addition, some protein hydrolysates are very hygroscopic, hydrophobic, reactive, and allergenic. Microencapsulation may be an alternative for reducing these problems. For many years, this technique has been used in the pharmaceutical industry for time-release and enhanced stability of formulations and flavor masking. Moreover, microencapsulation provides a useful technique for protecting products from environmental conditions to extend shelf-life (Shahidi & Han, 1993) and as nutraceutical delivery systems through the incorporation of bioactive compounds into food systems (Chen et al., 2006).

Hyprol is a hydrolyzed enzymatic digest of casein providing a high quality source of peptides, including casein phosphorpeptides. This product is recommended as a soluble peptide source in enteral nutrition, infant food formulas, and protein enrichment of food and beverages (Kerry Bio-Science, 2005). Despite these properties, Hyprol presents an intense bitter taste, which limits its direct addition as a food ingredient.

Soy protein isolate (SPI) is produced from defatted soy meal by alkali extraction followed by acid precipitation (pH 4.5) (Choa, Park, Batt, & Thomas, 2007). It is an abundant, inexpensive and renewable raw material.

In this study, SPI was selected as wall material for Hyprol encapsulation. Advantages of choosing food protein matrices include their high nutritional value, abundant renewable sources, and acceptability as a naturally occurring food component that is degradable by digestive enzymes (Chen et al., 2006). SPI has many

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useful functional properties for encapsulation, such as emulsification, solubility, film-forming and water binding capacity, in addition to high nutritional value and their generally recognized safe (GRAS) attributes (Fennema, 2000; Rhima). These properties make SPI a very attractive wall material for the encapsulation of bioactive compounds, however it is difficult to use in encapsulation molecules with the same biochemical structure in both wall and core. Moreover, the use of soy protein as wall material has been little explored.

The aim of this research was to study the potential of SPI as an encapsulation carrier of a casein hydrolysate and to evaluate the efficiency of the encapsulation process for reducing the bitter taste of casein hydrolysate.

2. Material and methods

2.1. Materials

Soy protein isolate (SPI) (Supro 500L, Solae Company, Brazil) was used as the encapsulating agent. According to the data supplied by the manufacturer this product has at least 90 g/100 g of protein (dry basis).

The core or active material used was casein hydrolysate (Hyprol 8052, Kerry Bio-Science, Holland), and its composition and amino acid profile (data supplied by the manufacturer) are shown in Fig. 1.

2.2. Microencapsulation by spray drying

2.2.1. Dispersion preparation

Dispersions with 12 g/100 g solids were prepared, using 70 or 80 g/100 g of SPI and 30 or 20 g/100 g of casein hydrolysate (treatments M1 and M2). Initially, the SPI was solubilized with magnetic stirring, and the pH was adjusted to 8.0. Then it was mixed with the casein hydrolysate previously solubilized. Next, the solutions were homogenized using a dispenser or homogenizer (Ultraturrax, TE-102, Tecnal, Brazil) for 2 min at 18,000 rpm. The samples were diluted 1:1.33 in distilled water before spray drying.

2.2.2. Spray drying

The dispersions were spray dried using a SD 5.0 (Labmaq, Brazil) pilot scale spray dryer. The dispersion was fed by a peristaltic pump at a fixed rate of 3.6 L/h. Drying was carried out in the concurrent mode. The dispersions at room temperature (23–25 °C) were atomized using 50 L/min compressed air. Inlet and outlet air temperatures were 140 °C and 110 °C, respectively. The microcapsule powder was collected at the bottom of the dryer's cyclone.

The products were stored in glass bottles, which were maintained at room temperature, in a dry place, in the absence of light.

The processing was carried out in three replicates. After each process, all the analyses were repeated in triplicate, except for morphology and sensory evaluation.

2.3. Microcapsule characterization

2.3.1. Moisture

The moisture content of the microcapsules was determined using a moisture analyzer, Ohaus, MB35 (USA).

2.3.2. Solubility

All samples (10 mg/ml solids) were dispersed in distilled water at pH 7.0 and constantly stirred for 1.5 h at room temperature and then centrifuged at 6000*g* for 5 min at 15 °C. Protein solubility was determined from supernatants using the Bradford method (Bradford, 1976) and was expressed as a concentration by mass, g of soluble protein per 100 g of total protein.

2.3.3. Hygroscopicity

For hygroscopicity, samples (about 2 g) of each powder were placed in Petri dishes at 25 °C in an airtight plastic container filled with Na_2SO_4 saturated solution (81% RH). After 1 week the samples were weighed and hygroscopicity was expressed as g of water absorbed/100 g of dry solids (Cai & Corke, 2000).

2.3.4. Water activity

Water activity was measured using an Aqualab analyzer (Decagon Devices, USA) at 25 °C after stabilization of the samples at this temperature for 1 h.

2.3.5. Particle size measurement

Sample diameter and size distribution were measured using laser light diffractometer equipment (Mastersizer X, Malvern Instrument, UK). The average particle size was expressed as the volume mean diameter in μm.

2.3.6. Morphology

The morphology of the microcapsules was observed with a scanning electron microscope (JEOL JSM–T300, Tokyo, Japan) at an accelerating voltage of 5 kV. Before using the scanning electron microscope, the samples were coated in argon atmosphere with gold/palladium using a Balzers evaporator (model SCD 050, Baltec Lichtenstein, Austria) (Oliveira, Moretti, Boschini, Baliero, Freitas & Favaro-Trindade, 2007).

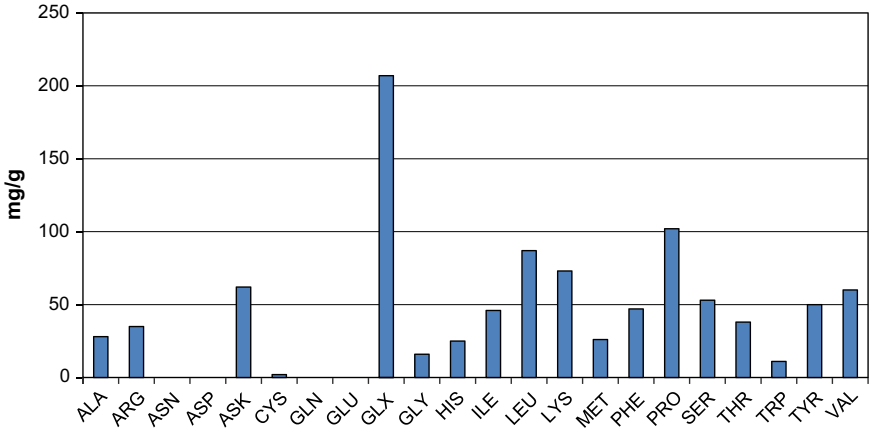

Fig. 1. Typical amino acid profile of Hyprol 8052 (mg/g). (Data from Kerry Bio-Science, 2005).

2.3.7. Hydrophobicity

The surface hydrophobicity was determined according to methods described by Hayakawa and Nakai (1985). The method was standardized by adjusting the intensity of the relative fluorescence at 80% of the scale by adding 15 μL of 8-aniline-1-naphthalensulphonate (ANS) 8 mmol/L to 3 ml pure methanol. The supernatants from the solubility test were used for this assay. The protein concentration was determined using the Lowry, Rosebrough, Farr, and Randall (1951) method. The protein solutions were diluted to concentrations between 1/10 and 1/1000 protein. The fluorescence was measured at excitation wavelength $\lambda_{excitation} = 364$ nm and emission wavelength $\lambda_{emission} = 475$ nm (digital fluorimeter, Perkin-Elmer model 2000). The required probe amount for saturation was 25 μL ANS 8 mmol/L for 3 ml of sample. Two series with 3 ml were prepared for each dilution, one as blank and the other with 25 μL ANS added. The fluorescence intensity for each protein concentration was determined by subtracting the fluorescence intensity of pure sample from the value of the sample with ANS. The slope of fluorescence intensity *vs*. protein concentration (g/100 g) plot was calculated by linear regression analysis and used as an index of the protein hydrophobicity (Ho).

2.3.8. Differential scanning calorimetry

A differential scanning calorimeter (DSC TA 2010) controlled by a TA 5000 module (TA Instruments, New Castle, DE, USA), with a quench cooling accessory was used to measure T_g. The samples were conditioned on hermetic aluminium pans and heated twice at 5 °C/min from 50 to 150 °C in an inert atmosphere (45 mL/min of N_2).

2.4. Sensory evaluation

A sensory comparison of the casein hydrolysate encapsulated and mechanically mixed with the wall material (in the same proportion) was done to evaluate the bitterness attenuation effect of the microencapsulation process.

The samples in powder form were submitted to two-sided paired-comparison tests, according to Meilgaard, Civille and Carr (1999). The sensory evaluation was carried out in laboratory scale individual booths under fluorescent white light, with 24 trained panelists, which were previously selected according to their acuity in perceiving the bitter taste.

A pair of samples (approximately 5 g each) was randomly served to the panel in white plastic cups coded with three digit random numbers. The panelists were asked to indicate the bitterest sample and were instructed to rinse their mouths with water between samples.

2.5. Statistical analysis

All data was statistically analysed by SAS (2001) (*Statistic Analysis System*), version 8.02, using the PROC ANOVA procedure. *Tukey's Honestly Significant Difference* (HSD) was adopted as the multiple comparisons procedure.

3. Results and discussion

This is the first study to evaluate the possibility of encapsulating casein hydrolysates using SPI as the wall material by spray drying with the aim of attenuating the bitter taste of casein hydrolysates.

3.1. Spray drying microencapsulation

The SPI solutions were adjusted to pH 8.0 to obtain the maximum solubility of the proteins. The total solid content of the dispersion used in this study was 12 g/100 g because at higher concentrations, the dispersions become highly viscous.

Table 1
Characteristics of encapsulated samples and free casein hydrolysate.

	Free casein hydrolysate	M1	M2
Solubility in water (g/100 g)	100 ± 5.1^a	86.7 ± 5.9^b	94.9 ± 1.1^a
Water activity	0.21 ± 0.001^b	0.31 ± 0.017^a	0.30 ± 0.001^a
Moisture (g/100 g)	4.38 ± 0.03^c	5.16 ± 0.24^b	5.64 ± 0.06^a
Hygroscopicity (g of water/100 g of dry solids)	53 ± 0.07^c	106.99 ± 0.06^a	102.19 ± 0.22^b

Different letters in the same line indicate a statistically significant difference between the values ($p < 0.05$).
M1: treatment with 70 g/100 g of SPI and 30 g/100 g of casein hydrolysate.
M2: treatment with 80 g/100 g of SPI and 20 g/100 g of casein hydrolysate.

3.2. Microcapsules characterization

The characterization of the encapsulated samples is shown in Table 1, where it is also compared to the properties of the free hydrolysate.

The encapsulation process increased water activity, moisture and hygroscopicity in both treatments (M1 and M2) and reduced significantly the solubility ($p < 0.05$) of casein hydrolysate in treatment M1.

The treatment M2 had significantly higher solubility than M1. Thus, the solubility decreased with the increase in casein hydrolysate concentration in the microcapsule composition. But, the treatment M1 was slightly more hygroscopic than M2. However the difference was very small and should not result in any important technological implications. It can be seen in Fig. 2 that there was no difference in water absorption kinetic behavior between the two samples. For both, the absorption was quite fast (1.75 g of water/h) and occurred within the first hour after starting the test.

The bitter taste of casein hydrolysate is directly related to the existence of hydrophobic groups and its attenuation (to be discussed below) suggests that, because of the encapsulating process, the hydrophobic groups were less exposed, probably due to hydrophobic interactions with the SPI. These interactions caused conformational changes which increased the amount of superficial hydrophilic groups increasing the water activity, the moisture, and

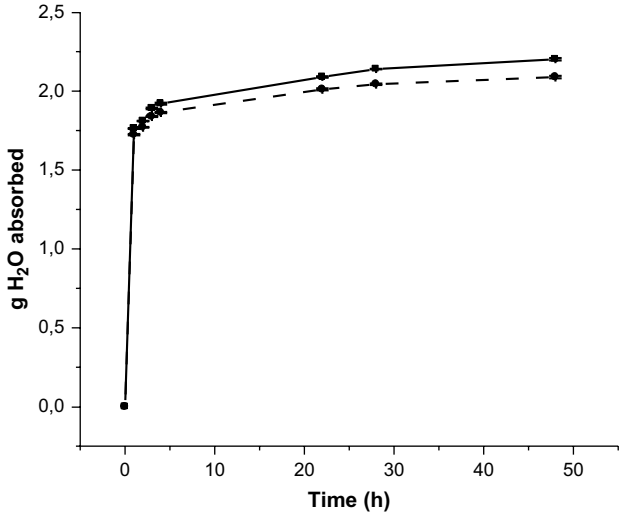

Fig. 2. Water absorption of encapsulated samples. M1 (■) and M2 (–•–). M1: treatment with 70 g/100 g of SPI and 30 g/100 g of casein hydrolysate. M2: treatment with 80 g/100 g of SPI and 20 g/100 g of casein hydrolysate.

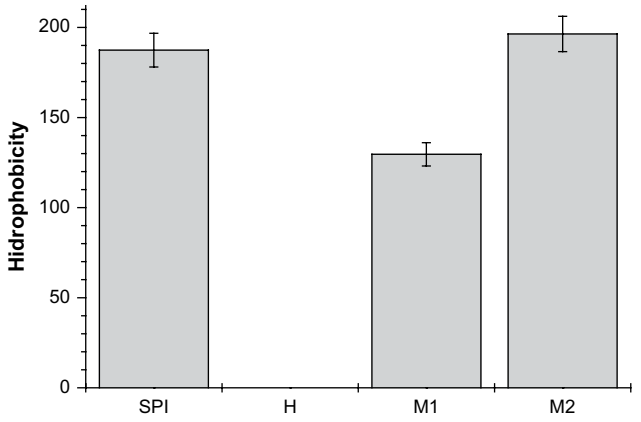

Fig. 3. Surface hydrophobicity of soy protein isolate (SPI), casein hydrolysate (H), and samples M1 and M2. M1: treatment with 70 g/100 g of SPI and 30 g/100 g of casein hydrolysate. M2: treatment with 80 g/100 g of SPI and 20 g/100 g of casein hydrolysate.

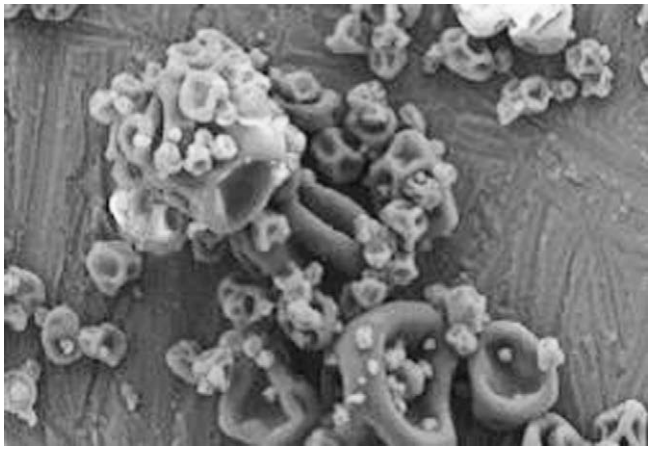

Fig. 4. Scanning electron micrographs of microcapsules (M1). Magnification of 5.000×.

the hygroscopicity of the encapsulated casein hydrolysate compared to the free one (Table 1).

As the surface hydrophobicity determination requires a hydrophobic zone within a "pocket"-like structure to link to the probe, it cannot be accomplished for casein hydrolysate. The low molecular weight of the peptides of the casein hydrolysate does not provide the necessary conformation for the analysis, apart from the high hydrophobicity, which the lateral chains of amino acids may have.

The encapsulated M1 had lower hydrophobicity than the SPI (Fig. 3). This result suggests that, during encapsulation, hydrophobic interactions occur between the SPI and the casein hydrolysate lessening the exposure of the surface hydrophobic groups. In the case of encapsulated M2, which had lower casein hydrolysate content, surface hydrophobicity was similar to that of SPI. The differences found in the surface hydrophobicity of encapsulates compared to the SPI indirectly demonstrate that the encapsulating process was accomplished.

The SEM revealed the absence of free material confirming the formation of microencapsulates of varied particle sizes (Fig. 4). The microcapsules produced with both treatments (M1 and M2) had similar appearance: were spherical, with concavities typical of spray dried particles. External surfaces showed walls with no fissures, cracks, or disruptions; this is fundamental for guaranteeing lower permeability of gases, higher protection and stuffing retention.

The microcapsules produced were very resistant to mechanical fracture. The authors were unable to fracture the material even after using the method described by Santos, Favaro-Trindade, and Grosso (2005) (i.e., liquid nitrogen aspersion followed by striking with a scalpel) and thus it was impossible to observe the internal morphology of the microcapsules. The use of protein as wall material allows a harder structure for the microcapsules, a phenomenon that was also reported by Oliveira, Moretti, Boschini, Baliero, Freitas, & Favaro-Trindade (2007), when producing microcapsules by complex coacervation using a casein/pectin complex as the wall material, followed by spray drying.

The structure observed (without cracks or disruptions) and its resistance also revealed the strong film-forming property of SPI.

Treatments M1 and M2 had an average particle size of 11.32 ± 0.09 and 9.18 ± 0.07 μm, respectively, and both presented a clear bimodal distribution (between 0.1 and 100 μm) (Fig. 5 a and b).

No data was found in the literature concerning the encapsulation of casein hydrolysate by spray drying. Some authors have encapsulated casein hydrolysates in liposomes [Morais et al., 2003; Morais, De Marco, Oliveira, & Silvestre, 2005] and lipospheres (Barbosa et al., 2004) and they also reported the decrease in hydrophobicity of casein hydrolysates after microencapsulation.

The results of the DSC analyses are presented in Fig. 6. The SPI showed a single endothermic peak at 111.83 °C, indicating partial soy protein denaturation caused by the dehydration process of

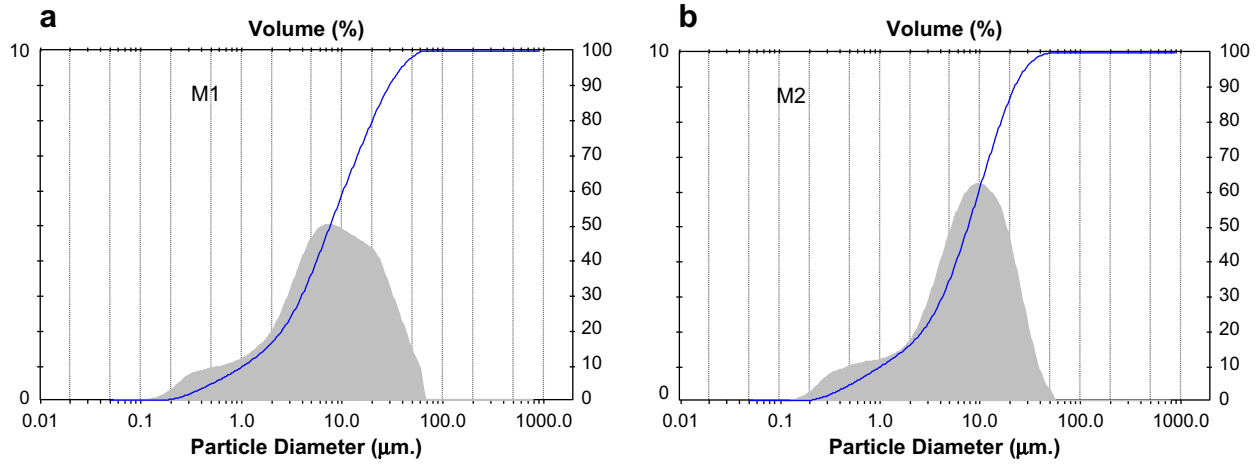

Fig. 5. a and b – Particle size distribution of M1 and M2 samples. M1: treatment with 70 g/100 g of SPI and 30 g/100 g of casein hydrolysate. M2: treatment with 80 g/100 g of SPI and 20 g/100 g of casein hydrolysate.

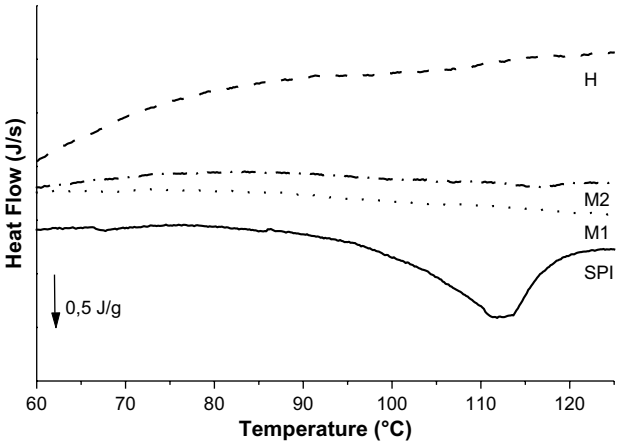

Fig. 6. DSC thermograms of soy protein isolate (SPI), casein hydrolysate (H), samples.

spray drying. Native soybean protein had two endothermic peaks at 75 and 85 °C corresponding to 7S and 11S globulins, which are the major fractions in the isolate.

As expected for the casein hydrolysate, due to its extended structure, no peaks occurred.

The absence of peaks in the thermograms of the encapsulated samples may indicate modification in their protein structure, as protein denaturation by heat during spray drying or the effect of process encapsulation may occur.

3.3. Sensory evaluation

According to Meilgaard et al. (1999), in a two-sided paired-comparison test with 5% significance level and 24 panelists, the response must contain a minimum of 17 panelists choosing one of the two samples to find a significant difference between them. Accordingly, encapsulation with SPI showed to be effective in attenuating the bitterness of the casein hydrolysate. For treatments M1 and M2, 18 and 17 panelists respectively, checked the encapsulated samples as tasting less bitter. It is likely that the microencapsulation process using SPI as the wall material reduced the exposure of hydrophobic amino acids, resulting in a less bitter product than the casein hydrolysate alone.

This result was similar to that obtained by Barbosa et al. (2004), Morais et al. (2003), and Morais et al. (2005), who found that the encapsulation of casein hydrolysates in liposomes and lipospheres caused bitterness reduction. However, spray drying is a low cost and simple process and soy protein is an abundant, inexpensive and renewable natural material. Besides, delivery devices based on soy proteins are remarkably promising because of their high nutritional value and excellent functional properties.

4. Conclusion

As demonstrated in this paper, the spray drying process and soybean protein isolates can be used to encapsulate casein hydrolysate, opening a new path for specific applications and the development of innovative delivery systems and/or functional food products.

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