Abstract

A total of 94 dry-cured white hams were purchased in the North-East of Spain in two years: 1995 and 2007, belonging to three different varieties: ‘Jamón Serrano’ Traditional Speciality Guaranteed with a minimum of 7 months of curing time (JS), a common non-labelled inexpensive dry-ham and less than 6 months of curing time (J6m) and ‘Jamón de Teruel’ Protected Designation of Origin with a minimum of 12 months of curing time (JT). From these samples, the leg was deboned and a slice of the Biceps femoris was obtained which was then vacuum packaged and kept frozen until the fatty acid composition was analyzed. Only those fatty acids detected in 1995 have been taken in consideration for the comparison in 2007. Significant interactions between the year of the survey and the type of ham appeared only in minor fatty acids. The type of ham influenced most fatty acids, with JT showing the highest proportion of C16:0 and the lowest of C18:2 \( n \)-6 and C18:3 \( n \)-3 \( (p<0.001) \) in both surveys. The highest percentage of oleic acid found in all hams in 2007 implied a significantly higher monounsaturated fatty acids composition in this year than in 1995. All types of hams and year of survey could be separated through a principal component analysis, which demonstrates the different quality of these hams in terms of fatty acid composition and that the dry-cured ham industry is in constant development towards a healthier fatty acid composition.

Additional key words: fatty acid development; intramuscular fat; Serrano ham; Teruel ham.

Resumen

Composición en ácidos grasos de variedades españolas de jamón curado. Muestreos de 1995 y 2007

Un total de 94 jamones de cerdo blanco fueron adquiridos en el Noreste de España en dos ocasiones: 1995 y 2007, procedentes de 3 variedades distintas: ‘Jamón Serrano’ Especialidad Tradicional Garantizada con un mínimo de 7 meses de curación (JS), un jamón común sin marca y económico de menos de 6 meses de curación (J6m) y ‘Jamón de Teruel’ Denominación de Origen Protegida con un mínimo de 12 meses de curación (JT). En todos ellos, se deshuesó el pernil y se obtuvo un filete del músculo Biceps femoris, que se envasó al vacío y congeló hasta que se realizaron los análisis de la composición en ácidos grasos. Sólo aquellos ácidos grasos detectados en 1995 se tuvieron en cuenta para la comparación en 2007. Interacciones significativas entre el año del muestreo y el tipo de jamón han aparecido sólo en ácidos grasos minoritarios. El tipo de jamón ha tenido una influencia significativa en la mayoría de ácidos grasos, siendo JT el que mayor proporción mostró de C16:0 y el que menor tuvo de C18:2 \( n \)-6 y C18:3 \( n \)-3 \( (p<0.001) \) en ambos muestreos. El mayor contenido en ácido oleico encontrado en todos los jamones en 2007 provocó significativamente un mayor contenido en ácidos grasos monoinsaturados en este año frente a 1995. Cada tipo de jamón y año de muestreo pudo separarse a través de un análisis de componentes principales, lo que demuestra la diferente calidad de cada uno de ellos en cuanto a composición en ácidos grasos se refiere, además de la constante evolución de la industria del jamón curado hacia un contenido más saludable de ácidos grasos.

Palabras clave adicionales: evolución de ácidos grasos; grasa intramuscular; jamón Serrano; jamón de Teruel.

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Abbreviations used: AI [(atherogenic index (C12:0+C14:0+C16:0)/(n-3 PUFA + n-6 PUFA + MUFA)), FA (fatty acids), FAMES (fatty acids methyl esters), IMF (intramuscular fat), I1 [(C18:0+C18:1)/C16:0], JS (Serrano ham), JT (Teruel ham), J6m (non labeled ham with less than 6 months of curing time), MUFA (monounsaturated fatty acids), PDO (Protected Designation of Origin), PUFA (polyunsaturated fatty acids), SFA (saturated fatty acids), TI [thrombogenic index (C14:0+C16:0+C18:0)/(3n-3 PUFA + 0.5n-6 PUFA + n-3 PUFA/n-6 PUFA)], TSG (traditional speciality guaranteed).
Introduction

Spain is a world leader in dry-cured ham, which is a traditional product consumed in the country. More than 41 million pieces were produced in 2007 while the consumption per capita is 2.4 kg per year (Cruz, 2009). Due to the current awareness of the nutritional properties of food, the composition of the fat from dry-cured ham may not be considered among the healthy fats in the human diet, because of its animal origin it is included among the meat that contains cholesterol, large amounts of saturated fatty acids (SFA) and low levels of n-3 polyunsaturated fatty acids (PUFA) (Rhee, 1992). However, the composition of meat fat depends on many factors, such as animal species, genetics and feeding.

Over the last few years production and technological conditions in the ham industry have greatly developed, which has benefited the commercialisation of supplying foreign demand. The manufacturing technology of dry-cured ham is in constant development (Cilla et al., 2005) due to the requirement of a final phase of maturation or ripening after drying. Focus from producers has been made on the study of technological aspects such as the length and conditions of ripening (Cilla et al., 2005) or temperature of storage (Cilla et al., 2006a). Whereas neither refrigeration nor freezing provokes sufficient modification of ham quality in order to modify the acceptance of consumers (Cilla et al., 2006a), the excessive length of the maturing process produces biochemical changes that result in quality deterioration as assessed by a trained panel (Cilla et al., 2005).

The genetic origin of the animals also highly influences some of the characteristics of the ham (Latorre et al., 2003). From the five Protected Designation of Origin (PDO) of dry-cured ham that exists in Spain, four of them are from Iberian pigs, and only ‘Jamón de Teruel’ is based on white pigs (crossbreeds from Duroc, Large White and Landrace). As a sire line Duroc is accepted in the PDO ‘Jamón de Teruel’ (BOA, 1993), with the aim of increasing the fat percentage (Edwards et al., 1992; Alonso et al., 2009). The crossbred sows between Landrace and Large White that have been traditionally used may not show more than 2.7% of intramuscular fat (Olives et al., 2009) in contrast to the 4% that can be reached in Duroc or Iberian pigs (Daza et al., 2007a). However, there is also a large variability within the sire line even when using the same breed which can affect carcass and meat quality parameters (Cilla et al., 2006b). Other production factors, such as gender or weight at slaughter, have also been studied (Latorre et al., 2009a,b).

Although the composition of the feed and the intake level at the fattening phase can also alter the composition of the fat, there are fewer studies about these variables in relation to the quality of the final product in white pigs than those that can be found in Iberian pigs, where the feeding regime is highly related to the role of the fat in the quality of the ham (Daza et al., 2007b, 2008; Dunker et al., 2007; Olivares et al., 2009). Analytical techniques have also been developed to avoid frauds (López-Vidal et al., 2008) due to different prices of the final product in relation to the production costs and quality characteristics. In hams from white pigs, the shortage of references might be the result of the focus of the production chain on genetics and technological conditions during ripening in order to improve the quality of the ham offered to the consumer. Due to the obvious evolution of the dry-cured ham industry, the aim of this work was to assess an important variable in the quality of ham, such as the fatty acid composition, focusing in their development throughout the years in commercial hams from ‘Jamón de Teruel’ PDO, and to compare them with some competitors in the market, whose origin is also from white pigs, taking into account the usefulness and difficulties of studies with data in different periods (Ngapo and Dransfield, 2006).

Material and methods

Sampling

A certain number of dry-cured white hams were purchased in different marketplaces in Zaragoza (North East of Spain) in two periods of time: 1995 and 2007. Sixteen hams of each of the following varieties were analyzed in 1995: ‘Jamón Serrano’ TSG (JS) (dry-ham produced in Baza in the South East of Spain with a minimum of 7 months of curing time), a common non-labelled dry-ham with an inexpensive price at the retailer and under 6 months of maturity (J6m) and ‘Jamón de Teruel’ PDO (JT) (dry-ham produced in the region of Teruel in the North East of Spain with a minimum of 12 months of curing time). In 2007, 8 hams were analyzed of JS, 13 of J6m and 25 of JT. In each survey year, JS and JT hams were manufactured by the same company. J6m hams were purchased in the same market in each survey. For all hams, the leg was deboned...
and a 1 cm-thick slice obtained, containing the muscle *Biceps femoris* on its central part. Then, this muscle was excised, vacuum packaged, frozen and kept at −18°C until analyzed.

**Lipid extraction**

Prior to the extraction, samples were thawed maintaining the vacuum packaging. The muscle was grounded and the fat from 10 g of *Biceps femoris* was extracted in chloroform-methanol, according to Bligh and Dyer (1959) and 2,6-di-ter-butyl-4-methylphenol (BHT) from Panreac® (1 g/10 mL methanol) was used as antioxidant (Carrilho *et al*., 2009). Fatty acid methyl esters (FAMES) were generated by transesterification of 30 mg of the lipid extracts dissolved in n-hexane (2 mL) with 2 N KOH in methanol at room temperature. FAMES were collected in hexane for analysis by gas chromatography.

**Gas chromatography analysis of fatty acid methyl esters**

Due to the improvement of the equipment, different gas chromatographs were used in the analysis of 1995 and 2007. In 1995, the fatty acid composition was determined by gas chromatography with a Hewlett-Packard 5890 Series II equipped with a capillary column MFE1000 (25 m × 0.25 mm × 0.25 µm) and oven temperature programming as follows: column temperature was set at 180°C, then raised at a rate of 3°C min⁻¹ up to 210°C, kept for 5 min and raised at 5°C min⁻¹ to 225°C, and kept constant for 9 min. Inlet temperature was kept at 230°C and the detector at 240°C. A split mode injector with split ratio of 1/30 was applied. Nitrogen was used as a gas carrier at a constant flow rate of 0.8 mL min⁻¹ with an injected volume of 1 µL. In both surveys, the methyl esters were identified using retention times of Sigma chemical Co. Standards and some fatty acids indexes were calculated: I₁ ((C18:0+C18:1)/C16:0), AI ((C12:0+C14:0+C16:0)/(n-3 PUFA + n-6 PUFA + MUFA) and TI ([C14:0+C16:0+C18:0)/(3n-3 PUFA + 0.5n-6 PUFA + n-3 PUFA/n-6 PUFA]) (Banskalieva *et al*., 2000; De Lorenzo *et al*., 2001).

**Statistical analysis**

Due to the higher separation and detection of the equipment used in 2007, only those fatty acids identified in 1995 have been considered in 2007, normalising their percentage to the same total percentage for both to facilitate comparison of results. A general linear model (GLM) (SAS, 1994) has been used to assess the effect of year of survey, type of ham and their interactions on the percentage of individual and groups of fatty acids. When significant, a Duncan test has been used to assess differences between mean values. A principal component analysis (PCA) was used to study the sample’s variability and the relationship among the variables and treatments studied.

**Results**

Table 1 shows the data of the individual fatty acids. Significant interactions between the year of the survey and the type of ham have appeared in minor fatty acids (with a percentage below 1% of the total fatty acid profile): C15:0, C20:0 and C20:4 n-6. An influence of the year of the survey has been found in C12:0, C14:0, C18:2 n-6 and, especially (*p < 0.001*), in C18:1 n-9, C18:3 n-6, C20:0 and C20:4 n-6. The higher percentage of oleic acid (C18:1 n-9) found in all hams in 2007 (*p < 0.001*) implied a significant higher MUFA in 2007 than in 1995 (*p < 0.01*) (Table 2). However, this did not correspond with significant differences in the saturation of the intramuscular fat (IMF), since there were no differences in individual SFA except for C12:0, C14:0 and C20:0. The lower proportion of PUFA (*p < 0.001*) in 2007 was mainly due to the lower content of n-6 PUFA. The year of the survey did not have a significant effect in the percentage of saturated fatty acids, n-3 PUFA, the ratio n-6/n-3, and the indexes I₁ and AI.
The type of ham has influenced the percentages of most fatty acids except for C12:0, C18:0, C18:1 n-7 and C20:0. In both surveys, JT has shown the highest proportion of C16:0 (p < 0.001) and the lowest of C18:2 n-6 and C18:3 n-3 (p < 0.001). The differences in palmitoleic and oleic acids in 1995, with the highest content found in JT in relation to the other types, disappeared in 2007, where no differences were found between the hams. On the other hand, although no differences were found in 1995, JT showed higher percentages of C14:0 and lower of C18:3 n-6 and C20:4 n-6 than the other two types in 2007.

In the groups of fatty acids, no interaction was found between year of survey and type of ham. The type of ham was important for all variables except for the ratio n-6/n-3. In both surveys, JT showed a more saturated fat than J6m and a lower percentage of PUFA than the other two types, especially due to the lower percentage of n-6 PUFA. As a result, the ratio PUFA/SFA was significantly lower in JT than in JS and J6m. Although

Table 1. Percentage of identified fatty acids in the intramuscular fat of m. Biceps femoris in dry cured ham

|       | JS n = 16 | J6m n = 16 | JT n = 16 | JS n = 8 | J6m n = 13 | JT n = 25 |
|-------|-----------|------------|-----------|----------|------------|----------|
| C12:0 | 0.09 ± 0.01 | 0.11 ± 0.04 | 0.10 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.02 | 0.09 ± 0.01 |
| C14:0 | 1.43 ± 0.16 | 1.42 ± 0.16 | 1.49 ± 0.19 | 1.35 ± 0.12 | 1.36 ± 0.10 | 1.43 ± 0.14 |
| C15:0 | 0.06 ± 0.03 | 0.06 ± 0.02 | 0.05 ± 0.02 | 0.08 ± 0.06 | 0.04 ± 0.01 | 0.04 ± 0.01 |
| C16:0 | 23.04 ± 1.30 | 22.75 ± 1.32 | 23.98 ± 1.39 | 23.37 ± 1.16 | 22.75 ± 1.32 | 24.24 ± 0.98 |
| C16:1 | 3.41 ± 0.59 | 3.42 ± 0.59 | 3.75 ± 0.56 | 3.66 ± 0.47 | 3.43 ± 0.37 | 3.70 ± 0.53 |
| C17:0 | 0.30 ± 0.09 | 0.27 ± 0.06 | 0.22 ± 0.04 | 0.35 ± 0.06 | 0.27 ± 0.05 | 0.22 ± 0.04 |
| C18:0 | 11.55 ± 1.15 | 11.19 ± 1.04 | 11.39 ± 1.27 | 11.37 ± 0.91 | 10.98 ± 0.91 | 11.65 ± 1.10 |
| C18:1 n-9 | 44.79 ± 1.85 | 44.51 ± 2.26 | 45.04 ± 1.69 | 45.72 ± 1.61 | 46.67 ± 1.78 | 46.56 ± 1.56 |
| C18:1 n-7 | 4.58 ± 0.52 | 4.56 ± 0.42 | 4.77 ± 0.49 | 4.69 ± 0.25 | 4.47 ± 0.21 | 4.47 ± 0.43 |
| C18:2 n-6 | 8.93 ± 2.43 | 9.95 ± 2.85 | 6.63 ± 1.25 | 7.82 ± 1.47 | 8.34 ± 1.97 | 6.50 ± 1.24 |
| C18:3 n-6 | 0.11 ± 0.02 | 0.10 ± 0.03 | 0.09 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| C18:3 n-3 | 0.56 ± 0.17 | 0.56 ± 0.17 | 0.44 ± 0.09 | 0.48 ± 0.10 | 0.54 ± 0.15 | 0.41 ± 0.07 |
| C20:0 | 0.23 ± 0.08 | 0.21 ± 0.04 | 0.17 ± 0.04 | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.17 ± 0.03 |
| C20:4 n-6 | 0.90 ± 0.12 | 0.86 ± 0.07 | 0.86 ± 0.09 | 0.90 ± 0.39 | 0.84 ± 0.55 | 0.49 ± 0.23 |

ns: not significant. *: p ≤ 0.05. **: p ≤ 0.01. ***: p ≤ 0.001. a,b,c: mean values within year in the same row with different letters differ significantly (p ≤ 0.05).

Table 2. Percentage of groups of identified fatty acids in the intramuscular fat of m. Biceps femoris in dry cured ham

|       | JS 1995 | J6m 1995 | JT 1995 | JS 2007 | J6m 2007 | JT 2007 |
|-------|---------|---------|--------|---------|---------|--------|
| SFA   | 36.71 ± 2.12 | 36.02 ± 1.90 | 37.40 ± 2.26 | 36.77 ± 1.69 | 35.67 ± 2.09 | 37.85 ± 1.71 |
| MUFA  | 52.79 ± 2.71 | 52.49 ± 3.00 | 54.57 ± 1.83 | 54.08 ± 1.30 | 54.56 ± 1.90 | 54.73 ± 1.95 |
| PUFA  | 10.50 ± 2.60 | 11.48 ± 3.04 | 8.02 ± 1.18 | 9.14 ± 1.81 | 9.76 ± 2.54 | 7.42 ± 1.43 |
| n-3 FA | 0.56 ± 0.17 | 0.56 ± 0.17 | 0.44 ± 0.09 | 0.48 ± 0.10 | 0.54 ± 0.15 | 0.41 ± 0.07 |
| n-6 FA | 9.94 ± 2.46 | 10.92 ± 2.90 | 7.59 ± 1.11 | 8.66 ± 1.79 | 9.22 ± 2.45 | 7.02 ± 1.37 |
| PUFA/SFA | 0.21 ± 0.06 | 0.22 ± 0.07 | 0.15 ± 0.02 | 0.17 ± 0.03 | 0.18 ± 0.05 | 0.13 ± 0.03 |
| n-6/n-3 | 18.30 ± 2.84 | 20.02 ± 4.22 | 17.62 ± 2.63 | 18.64 ± 4.70 | 17.57 ± 3.61 | 17.36 ± 1.95 |
| I1 4  | 2.45 ± 0.15 | 2.46 ± 0.16 | 2.40 ± 0.17 | 2.45 ± 0.18 | 2.54 ± 0.18 | 2.40 ± 0.13 |
| AI 5  | 0.39 ± 0.03 | 0.38 ± 0.03 | 0.41 ± 0.04 | 0.39 ± 0.03 | 0.37 ± 0.03 | 0.41 ± 0.03 |
| TI 6  | 5.79 ± 1.80 | 5.29 ± 1.52 | 7.33 ± 1.41 | 6.46 ± 1.75 | 5.93 ± 1.61 | 8.09 ± 1.70 |

1 SF: saturated fatty acids. 2 MUFA: monounsaturated fatty acids. 3 PUFA: polyunsaturated fatty acids. 4 I1: (C18:0+C18:1)/C16:0. 5 AI: (C12:0+C14:0+C16:0)/(n-3 PUFA + n-6 PUFA + MUFA). 6 TI: (C14:0+C16:0+C18:0)/(3 n-3 PUFA + 0.5 n-6 PUFA + n-3 PUFA/n-6 PUFA). ns: not significant. *: p ≤ 0.05. **: p ≤ 0.01. ***: p ≤ 0.001. a,b: mean values within year in the same row with different letters differ significantly (p ≤ 0.05).
n-3 FA also showed lower values in JT, these were no different from JS in 2007. The atherogenic and thrombogenic indexes showed lower values in J6m than JT, whereas I1 was not different between the types of ham in 1995, turning higher in J6m in 2007 in relation to JT.

The PCA results are shown in Figure 1. The first two PCs explained 80.46% of the variability. PC1 clearly separated the types of ham, especially the less cured (J6m), placed positively, from the most cured (JT), and located in the negative part of the axis. PC2 clearly separated the year of the survey; hams surveyed in 1995 were placed in the positive part of the scale, and those surveyed in 2007 in the negative part of the graph. Variables were mostly separated by PCA1, where PUFA, PUFA/SFA, n-6 FA, n-3 FA and essential FA such as C18:2 n-6 and C18:3 n-3 were located away from the origin and close to the axis in the positive part of the scale, negatively correlated with C16:0, C16:1, T1, AI, SFA and MUFA, that were placed in the negative part of the axis. J6m was mostly characterized by PUFA, JT by SFA and JS was placed between the other two groups.

Discussion

Although differences between types of ham have been more pronounced than between the two surveys, at least in the groups of fatty acids, the significant differences found in 2007 related to 1995 imply that the pork industry is in constant development, trying to improve and adapt the quality of its product to the consumer. Not only well-known hams, such as JT or JS, are subjected to evolve due to the manufacture industry taking care of production aspects such as the genetic line or the feeding, but producers of ham without a quality label and with a short ripening period (J6m) are also developing a better product in terms of fatty acid composition. All studied hams showed higher MUFA composition in 2007 than in 1995, due to the increase in the percentage of oleic acid. MUFA and PUFA in human diets have been observed to decrease cholesterol levels in blood and to favour low incidence of cardiovascular diseases (Mattson and Grundy, 1985). The levels found in this study are lower but not too far from those found in Iberian ham, with approximately 49% of C18:1 n-9 (Fernández et al., 2007), considered a healthy product in the diet (García-Rebollo et al., 1998). In fact, the oxidation of unsaturated fatty acids, especially oleic acid, can form volatile compounds that can differentiate Iberian hams and JT from other hams of different geographical origin and different ingredients in the feeding (Luna et al., 2006). This increase in MUFA may have appeared as a consequence of the change in the diet of the animal reducing the incorporation of saturated fats in the feed, or to the inclusion
of Duroc as a predominant sire line (Cameron and Enser, 1991).

Alonso et al. (2009) found significant differences in the intramuscular profile according to different sire lines at the same level of fatness. Thus, Duroc showed higher percentage of palmitic and stearic acid and lower of PUFA than Pietrain. The common use of this sire line in quality hams that want to improve its content in IMF may have contributed to the observed MUFA and PUFA percentages. However, the lower percentage of PUFA found in JT might also be due to its higher IMF content in relation to JS and J6m (Sierra et al., 1998). Higher fatness implies higher composition of saturated fat due to the accumulation in the neutral lipid fraction, and JT showed a higher composition in SFA than J6m in both surveys. Nevertheless, the length of ripening can also affect the content of PUFA, with a reduction during maturation due to biochemical changes and enzymatic action in the polar lipid fraction (Cava et al., 2003). Since JT is the ham with the longest ripening with 12 months at least (BOA, 1993, 2009) versus 7 months at least for JS, the extra duration of the process may have contributed to a stronger decrease in the content, and therefore in the percentage of PUFA. Also, the hydrolysis during processing would have increased the amount of free fatty acids, which together with lypolisis are the two major processes that cause deterioration in the quality of meat during both storage and processing (Morrissey et al., 1998).

Some ratios among fatty acids are used to assess the nutritional properties of a product, in terms of human health. Among them, PUFA/SFA and n-6/n-3 are widely used. Values of PUFA/SFA above 0.4 and of n-6/n-3 below 4 are recommended (UK Department of Health, 1994). According to these recommendations, dry-cured ham would not be within the desirable limits, not even ham from Iberian pigs (Fernández et al., 2007). The lower PUFA/SFA ratio found in all hams in 2007 (p ≤ 0.01) is probably the result of the increase in fatness. However, this did not correspond to a higher saturation but to a decrease in PUFA due to an increase in MUFA, which would keep the unsaturation of the muscle constant. It has been previously described a negative correlation between C18:1 (main contributor to MUFA) and C18:2 (Morgan et al., 1992) probably due to the inhibition of the enzyme Δ 9-desaturase, responsible for oleic acid synthesis (Jeffcoat and James, 1984). Some other indexes have also shown differences among types of ham (Table 2). Although no changes have been shown in I1 for JT and JS, J6m had a higher value in 2007, different from JT, due to the lack of increase in C16:0 in J6m, which is a leaner ham than the other two studied types. The lower PUFA content has caused a higher atherogenic and thrombogenic indexes in JT than in the other types.

The plot of the PCA (Fig. 1) confirms the development of the quality of the different hams in terms of fatty acid composition. Whereas JT was a ham of higher quality compared to others in 1995, JS has evolved towards a more similar FA profile and seems closer in 2007 to JT than it was in 1995. Fernández et al. (2007) also were able to separate JT from JS on the plot of a canonical discriminant analysis. ‘Jamón de Teruel’ was the first ham with a PDO recognised by the European Union, which confirmed at the time a quality standard and distinguishing production systems from the rest of the white hams, and therefore, it was the reference for other quality labels in the way of improving their product. The evolution of JT towards higher fatness content characterises this product with more SFA than JS or J6m. However, it is clearly reflected in all hams that the effort in the improvement of the quality in 2007 has been related to the composition of C18:1 n-9 and MUFA. Therefore, dry-cured ham produced nowadays guarantees to the consumer certain levels of healthy fatty acids that could not be found in ham in previous years.

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