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Chilled orange juices stabilized by centrifugation and differential heat treatments applied to low pulp and pulpy fractions

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ABSTRACT

Thermal treatments of citrus juices to inactivate microorganisms and pectinmethylesterase (PME) must be as soft as possible to preserve fresh taste. PME, a cell wall enzyme associated with pulp, is more heat resistant than spoilage microorganisms. This paper analyzes fresh taste and storage stability of orange juices in which the pulp receives a heat treatment more intense than the rest of the juice. The products compared where: A) low pulp juice treated at 60 °C–15 s; B) aseptic blend of A and the corresponding pulpy fraction treated at 85 °C–15 s; C) non aseptic blend of non-treated low pulp fraction and the corresponding pulpy fraction treated at 85 °C–15 s. Product C was finally treated at 60 °C–15 s and packed under aseptic conditions. PME activity in A, B and C was around 10% of that in the original juice (1.30 nkat/ml).
After 12 months at 3 °C, juices B and C retained the original fresh taste with minimal losses of color and cloudiness. Juice A maintained fresh taste but its color and cloud were not satisfactory.
Industrial Relevance: The procedures proposed in this paper can be applied to produce chilled orange juices without losses in fresh taste having in addition, higher stability than commercial juices. Other advantages when compared to non thermal emergent technologies are that can be carried out by regular equipments used in citrus industries with an important energy saving. These procedures imply energy savings of 22% to 38% when compared to thermal requirements of juice pasteurization at 85 °C.

1. Introduction

Thermal processes induce changes in food constituents, producing off-flavours that are more intense in heat-sensitive products such as citrus juices, especially when the treatments are severe. Thus, in high quality chilled citrus juices the thermal process is adjusted to minimize flavour alterations. These juices have better sensory characteristics than sterilized products stored at room temperature but, in any case, their flavour differs from that of the original fresh juice.

The goal of thermal treatment applied to citrus juices is to cause microbial and enzyme inactivation. For juices with low pH (around 3.5), enough microbial inactivation for short life high quality chilled juices can be achieved by mild treatments; in a clementine juice inoculated with *Lactobacillus plantarum*, a reduction higher than 5 log cycles was achieved by heat treatment at 57.5 °C for 20 s and no counts were found after a treatment at 60 °C for 10 s (Torres, Bayarri, Sampedro, Martinez, & Carbonell, 2008). As Sentandreu, Carbonell, Carbonell, and Izquierdo (2005) showed, juices treated at this temperature for 15 s did not differ in sensory acceptance from fresh juices. Nevertheless, this treatment does not inactivate pectinmethylesterases (PME), responsible of the cloud loss in orange juices.

PME is not a unique enzyme but a mixture of isoenzymes; Versteeg (1979) identified twelve forms of PME in citrus fruits, with very different heat stabilities. He called 'thermostable PME activity' the activity remaining after a treatment at 70 °C during 5 min, enough to cause cloud losses during the refrigerated storage. From this the importance of a sufficient inactivation of these isoenzymes, although temperatures higher than 70 °C cause thermal damage and losses in acceptance of orange juices (Sentandreu et al., 2005).

PME are cell wall enzymes located in the pulp of oranges (Rouse, 1977). By centrifugation of a citrus juice two fractions are obtained, a pulpy fraction with most of the PME activity and a low pulp fraction almost free of enzymatic activity. A mild thermal treatment, just enough to inactivate microorganisms, can be applied to the low pulp fraction whereas the pulpy fraction needs a more drastic treatment to inactivate PME. Finally, both fractions can be blended to reconstruct the juice. Based on this idea, our research team patented a procedure (Izquierdo, Carbonell, Sentandreu, Sendra, & Navarro, 2010) where after thermal treatments, both fractions are aseptically cooled, stored, blended and packed. The process was applied to a Clementine juice with the following operating conditions: low pulp fraction, 78% v/v of the original juice, treated at 60 °C for 15 s; pulpy fraction, 22% v/v of the original juice, treated at 85 °C for 15 s. After blending both fractions, the product had a residual PME of 2.6% and its taste was

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undistinguishable from that of the original fresh juice (Torres et al., 2008). According to Irwe and Olsson (1994), a remaining PME activity lower than 10% of the initial value is satisfactory for short life chilled products.

Since aseptic handling and blending are delicate operations in most of juice factories, a modification of the process is proposed in this work, by which, after centrifugation of fresh juice, only the pulpy fraction is thermally treated (85 °C for 15 s) and lately blended with the non-heated low pulpy fraction. Finally, the reconstituted juice is treated at 60 °C for 15 s to inactivate microorganisms immediately before packaging. This procedure avoids aseptic manipulation but pulpy fraction is treated twice (firstly at 85 °C for 15 s and at 60 °C for 15 s after the mixture). Furthermore, the low pulp fraction can also be considered as a final product by itself, appreciated by the segment of population that like juices with low pulp content.

The aim of this work was to study the differences in PME activity, color, cloudiness and fresh taste of the following juices:

- product A, just constituted by the low pulp fraction treated at 60 °C for 15 s.
- product B, an aseptic blend of A with the corresponding pulpy fraction treated at 85 °C for 15 s.
- product C, a non aseptic blend of the non-treated low pulp fraction and the corresponding pulpy fraction treated at 85 °C for 15 s. The resulting juice was treated at 60 °C for 15 s.

All juices were maintained at 3 °C to evaluate their stability along a year of storage.

2. Material and methods

2.1. Sample preparation

Lane Late oranges (*Citrus sinensis* L. Osb.) were harvested in May 2009, when their maturity index overcame the value of 15 (to avoid the bitter taste caused by its high limonin content in non ripe fruits), in Lliria (Valencia, Spain). The next day, fruits were washed in tap water, drained, sized and squeezed in an industrial extractor with five finger cups (model Exzel from Luzzysa, El Puig, Valencia, Spain) in our pilot plant. The raw juice was sieved (0.4 mm Ø, in a paddle finisher (Luzzysa), and homogenized at 20 MPa with a Manton–Gaulin pilot homogenizer (model 15M8TBA). Then, the juice was centrifuged at 10,000 rpm in a Westfalia separator (model SAOH 205) using a flow rate of 1 L/min and pulp discharges each 5 min giving a low pulp fraction computing the 84% of total volume, being the pulpy fraction the remaining 16%. This partition of volumes was selected to get a minimum proportion of pulpy fraction but enough fluid to be pumped through plate heat exchangers.

The resulting low pulp and pulpy fractions were used to prepare products A, B and C as detailed below. Fig. 1 summarizes the processes applied to obtain these three products.

To prepare product A, the low pulp fraction obtained by centrifugation was pasteurized at 60 °C for 15 s in a plate heat exchanger (model Junior, APV Ibérica, S.A., Madrid, Spain), cooled at 7 °C in the last section of the heat exchanger and aseptically packed in 946 ml (1/4 gal) glass jars with twist-off cups previously sterilized with fluent steam.

To prepare product B, the pulpy fraction was pasteurized at 85 °C for 15 s and cooled at 7 °C. The low pulp fraction was pasteurized at 60 °C for 15 s and cooled at 7 °C. Aseptic blending was carried out dosing in each glass jar the corresponding amounts of both fractions. Jars were closed and shacked for mixing.

Product C was prepared by blending under hygienic conditions the pulpy fraction pasteurized at 85 °C for 15 s with the non-heated low pulp fraction. The final product was pasteurized at 60 °C for 15 s, cooled at 7 °C and bottled in glass jars as described for product A.

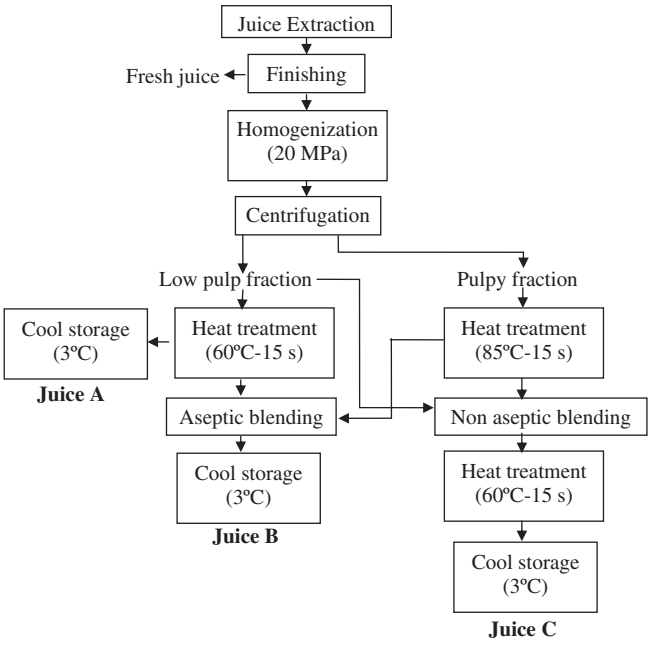

Fig. 1. Diagram of treatments applied to obtain the different orange juices.

Aliquots of each product were frozen immediately after bottling and stored at −20 °C and the remaining was stored at 3 °C for 2, 4, 6, 8, 10 and 12 months. After these periods of time, samples were frozen and stored at −20 °C until analyzed.

2.2. Brix, acidity, pH and essential oils

Total soluble solids were measured as °Brix with a digital refractometer (Pal-1, Atago Co. Ltd., Tokyo, Japan). Total acidity was assessed by titration with 0.1 N NaOH and expressed as % (w/v) of citric acid. The pH was determined by potentiometric measurement using a Crison GLP 21 pH-meter (Crison Inst. S. A., Barcelona, Spain). Recoverable essential oils were determined by bromate titration according to Scott & Veldhuis, 1966. All assays were performed at 22 °C and values are the average of three replicates.

2.3. PME activity

PME activity was determined according to a previously published method (Carbonell, Contreras, Carbonell, & Navarro, 2006) using a modification of the traditional procedure based on the titration of carboxylic groups generated by PME during the hydrolysis of a commercial pectin solution (Rouse & Atkins, 1955). Instead of measuring the volume of NaOH consumed to maintain pH at 7.8, pH decrease is recorded and fitted to a three parameters exponential decay equation. PME activity is calculated from the maximum value of the slope (at time zero) and expressed as nanokatals/ml. Values given are the average of three replicates.

2.4. Cloudiness and suspended pulp

10 ml of juice was poured in a graduated centrifuge tube with conical bottom and centrifuged at 22 °C for 10 min at 370 *g*. The supernatant was collected and its transmittance analyzed for cloudiness at 650 nm with a UV/visible spectrophotometer (Ultrospec 330 pro, Amaersham Bioscience, Pistacaway, NJ, USA). Juice clarification is evaluated according to the scale published by Cheng (2002): none, 0–24% of light transmission; slight, 25–35%; definite, 36–60%; and extreme, 61–100%.

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The suspended pulp was measured by centrifuging 10 ml of juice for 10 min at 3000 g in the same type of centrifuge tubes and reading the pulp volume. Suspended pulp was expressed as % (v/v). Values given are the average of three replicates.

2.5. Color

The color was measured with a Hunter colorimeter Labscan II model (Hunter Associates Lab., Reston, Vi, USA) controlled by a personal computer. An optical glass cells (3.8 cm high and 6 cm of diameter) containing a 3.5 cm thick layer of the juice sample was covered with the white standard plate (*X* 78.50; *Y* 83.32; *Z* 87.94) for measurement of diffused reflected light from the cell bottom using a 13 mm diaphragm aperture. Results were given in CIELAB system for illuminant D65 and a 10° angle of vision. The recorded parameters were: *L** (brightness), *a** (red component) and *b** (yellow component), calculating the Total Color Difference with respect to the original fresh juice through the equation

$$\Delta E = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^{*2}\right)\right]^{1/2}$$

Values given are the average of three replicates.

2.6. Sensory analysis

Sensory ranking tests (ISO, 2006) were carried out for: a) comparing the three products (A. B and C) at each storage time, b) comparing each sample with itself at different periods of storage (0, 4, 8 and 12 months). A group of assessors (37 to 54) with previous experience on sensory analysis of orange juice participated in the study. Each assessor was asked to rank samples according to the fresh taste, from the most intense fresh taste (rank number = 1) to the less intense fresh taste (rank number = n, if n samples were compared). 30 ml of samples were presented in white plastic cups coded with three digits random numbers. Mineral water was provided for mouth-rinsing. All sessions were carried out in a standardized test room (ISO, 2007) and data acquisition and analysis were performed using Compusense® five release 4.6 (Compusense Inc., Guelph, ON, Canada). Statistical analysis of sensory data consisted in adding up the rank order numbers assigned by the assessors to each sample. These sums of rank order numbers were analyzed for differences by the Friedman test (Meilgaard, Civille, & Carr, 1999).

3. Results and discussion

3.1. Physicochemical properties

The fresh Lane late juice had 10.3 °Brix and 0.66% of acidity, giving a ratio (Brix/Acidity) of 15.6, which corresponds to ripe oranges, very suitable for juice production; the pH was 3.49.

Table 1 shows the main characteristics of the different fractions and samples before storing the final products (juices named A, B and C). As expected, there was correspondence between sedimentable pulp and PME activity. There also was a significant relation between sedimentable pulp and essential oil. The essential oils, despite their lower density tended to accumulate with the pulp, as it was corroborated in previous assays with juices from different orange and mandarin cultivars (unpublished data); the concentration of essential oils in high pulp fractions was 4 to 7 times higher than that in the low pulp fractions. PME activity of final juices (0.14–0.15 nkat/ml) approached the maximum limit of 10% (0.13 nkat/ml in this case) recommended by Irwe and Olsson (1994). PME activity in fresh low pulp fraction, initially low, was slightly decreased by heat treatment at 60 °C for 15 s (0.19 vs 0.15 nkat/ml). By its hand, pulpy fraction where most of PME activity was accumulated (6.85 nkat/ml), was treated at higher temperatures (85 °C) to obtain juices (B and C) with low PME activities (0.14 nkat/ml). These results agree with those published in a former paper (Carbonell et al., 2006) where residual PME activities of 46.9% and 3.9% were measured after treatments at 63 °C for 10 s and 84 °C for 10 s respectively.

Table 1
Values[1] of PME, essential oil, transmittance and sedimentable pulp of different juice samples (see Fig. 1).

Sample	Sedimentable pulp, v/v (%)	Cloudiness (% transmittance at 650 nm)	Essential oil w/w (%)	PME (nkat/ml)
Fresh juice	7.0	19.25 ± 0.56	0.022 ± 0.002	1.30 ± 0.08
Fresh low pulp fraction	1.5	18.28 ± 0.39	0.011 ± 0.001	0.19 ± 0.02
Fresh pulpy fraction	14.5	0.41 ± 0.13	0.050 ± 0.004	6.83 ± 0.61
Low pulp juice treated at 60 °C–15 s (A)	1.5	2.91 ± 0.34	0.010 ± 0.001	0.15 ± 0.01
Blend of low pulp fraction treated at 60 °C–15 s and pulpy fraction treated at 85 C–15 s (B)	3.7	0.81 ± 0.12	0.019 ± 0.001	0.14 ± 0.02
Pulpy fraction treated at 85 °C–15 s was added to low pulp fraction; the blend was treated at 60 °C–15 s (C)	3.5	1.44 ± 0.15	0.019 ± 0.001	0.14 ± 0.01

[1] Average values ± standard deviation (3 replicates).

Regarding transmittance, all juices showed values lower than 24%, an adequate cloudiness for orange juices (Cheng, 2002). The transmittance of low pulp fraction was much lower after pasteurization (sample A, 2.91%) than before pasteurization (18.28%). A similar conclusion can be deduced comparing the transmittance of sample B (0.81%) and sample C (1.44%) with that of the fresh juice (19.25%). These results are consistent with those reported by Mizrahi and Berk (1970) who conclude that heat pasteurization of orange juice causes an increase in the number of fine particles at the expense of the coarser ones; the conversion of sedimentable pulp into coloidal pulp increases the cloudiness of juice. A similar result has been reported by Leizerson and Shimoni (2005) who gave a surface area mean diameter D[3,2] of 16.1 μm for fresh Shamuti juice and 3.6 μm for the same juice after a pasteurization at 90 °C for 50 s. This behavior could be attributed to the effect of mechanical stress during juice pasteurization on the particles in the juice.

The transmittance of the low pulp fraction (18.28%) is similar to that of the non fractionated fresh juice (19.25%), in spite to the homogenization treatment applied to the former. These results could be attributed to the different content in essential oils of the respective samples. The homogenization of a juice decreases the particle size in pulp, giving more cloudiness, but the further centrifugation removes not only sedimentable pulp but also approximately 50% of the essential oil of the juice. The droplets of essential oil dispersed in the juice are important components of the cloud (Baker & Bruemmer, 1969) and their decrease in the juice can counterbalance the effect of the reduction of the particle size in pulp.

Table 2 shows the changes in color CIELab parameters due to processing and to storage time and also the Total Color Differences with respect to the fresh juice. The differences with respect to the fresh juice were moderate in the full pulp juices (samples B and C) but very important in the low pulp juice (sample A), with a pronounced decrease of the three color parameters. Concerning storage, color was acceptably maintained by all samples. However, a slight decrease, mainly in brightness (L* parameter) and red color (a* parameter), was observed after 12 months of storage. Total Color Difference values

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Table 2
CIELab parameters of the different juices and Total Color Difference in relation to the fresh juice.

Storage time at 3 °C (months)	Juice A				Juice B				Juice C			
	L*	a*	b*	ΔE[1]	L*	a*	b*	ΔE[1]	L*	a*	b*	ΔE[1]
0	43.40	1.15	43.10	18.17	52.80	7.85	60.00	3.97	51.60	4.98	58.00	1.52
4	43.74	0.94	41.37	19.51	52.51	6.46	55.18	3.80	50.47	5.58	53.58	5.44
8	42.61	0.22	38.93	22.28	52.19	6.17	54.00	4.72	50.35	4.94	51.95	6.85
12	42.86	0.18	40.34	20.96	52.24	6.08	54.23	4.47	49.63	4.66	51.85	7.22

[1] ΔE with respect to the fresh juice (L* = 52.92, a* = 4.28, b* = 58.26).

seem to be stable with the storage time in juice B and increasing in juice C, but the variation is small; more analysis at different times and temperatures of storage would be necessary to confirm this behavior. Nienaber and Shellhammer (2001) studied the change in color of an orange juice coming from a citrus plant, processed at 800 MPa and 25 °C for 1 min and stored at 4 °C for 14 weeks; the authors fit the experimental points to a right line passing through the origin, but the fitted line was almost horizontal and makes an unequivocal conclusion difficult.

Fig. 2 shows the transmittance of juices A, B and C during 12 months of storage at 3 °C, following the experimental values of an exponential trend. The low pulp juice (sample A) quickly reached values of light transmission corresponding to an extreme level of clarification but the upper limit values (light transmission 25–35%) for samples B and C corresponded to a low level of clarification, what indicates an acceptable cloud maintenance. The transmittance increases initially in all samples, as a consequence of the residual PME activity (see Table 1), but after 3–4 months is stabilized. PME removes methoxy groups and produces free carboxylic radicals in pectin chains, a minor component of cloud which is important in maintaining the colloidal stability of orange juices. Then divalent cations (i.e. Ca^{+2}) cross-link carboxylic groups to other pectin chains giving macropolymers that tend to settle, entrapping other components of the cloud (Ackerley, Corredig, & Wicker, 2002; Ackerley & Wicker, 2003). Once the pectin has been precipitated, the colloidal components not dragged (Mizrahi & Berk, 1970) give a cloud less intense but stable at least during 12 months of refrigerated storage.

3.2. Sensory analysis

Differences in the intensity of perceived fresh taste among the three samples and differences due to the storage time were studied by different ranking tests. Table 3 shows the rank sums obtained when comparing the three samples at each period of storage whereas Table 4 shows the rank sums corresponding to the comparison of each sample with itself at different periods. These tables also show the values of the $F_{Friedman}$ for each comparison. Since the critical $F_{Friedman}$ values at the 95% confidence level are 5.99 for the comparison of 3 samples (Table 3) and 7.82 for the comparison of 4 samples (Table 4) it is concluded that sensory analysis did not show significant differences in anyone of the performed tests. That means on one hand, that at any time, fresh taste did not differ among products (Table 3) and, on the other hand, that fresh taste did not significantly decreased after 12 month of storage in any product (Table 4).

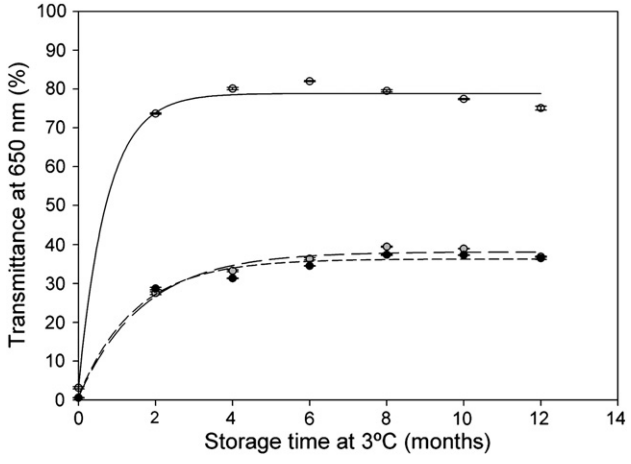

Fig. 2. Cloudiness evolution during the storage time of chilled citrus juices (white circles, juice A; gray circles, juice B; and black circles, juice C).

These results are important from two points of view, one related with the modification of the patented product B into product C and the other one with the low pulp juice, product A. Concerning the first aspect, it was previously observed (Torres et al., 2008) that the fresh taste of product B was not distinguishable from that of the original fresh juice. Thus, it can be concluded from the results of the present paper that the pulpy fraction can be pasteurized twice without losses in its fresh taste or, in other words, the modification of process B into process C, proposed for avoiding aseptic manipulation to reduce costs, is perfectly suitable. In relation to low pulp juice, product A, it can be concluded that pulp removal does not affect fresh taste.

It must be remarked that acceptability has not been tested, but only fresh taste. The main reason of this is that product A, poorer in pulp content, clearly differs in colour and texture from B and C. Thus, individual preferences of panellists to pulpy or non pulpy juices would condition their acceptability scores with the risk of giving confusing results. For instance, no global acceptability differences could have been concluded when, in fact, great differences, but in opposite senses could exist. To evaluate acceptability, a large number of panellists, representative of the potential consumers, is needed to identify segments of the population differing in preferences. This aspect will probably be approached in future works but, for the scope of this paper, we consider that "fresh taste" instead of "acceptability" was a better election since it has allow to confirm that, in spite of other differences between C and A (or B), an important attribute, fresh taste, presented a completely satisfactory level of intensity. Moreover, "fresh taste" was also suitable to compare products A and B since they only differed in the amount of possible heat damage that product B can accumulate due to have been pasteurized twice and heat affects mainly to fresh fragrance.

Table 3
Sensory comparisons of all products at each period of storage. Sums of rank order numbers for fresh taste.

Storage months	Juices			$F_{Friedman}$*
	A	B	C	
0	85	91	100	2.47
2	83	99	82	4.13
4	92	88	90	0.18
6	101	115	108	1.81
8	96	87	81	2.59
10	92	107	99	3.86
12	83	90	73	3.53

* Critical $F_{Friedman}$ value = 5.99 (95% confidence).

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Table 4
Sensory comparisons of each product with itself at different periods of storage. Sums of rank order numbers for fresh taste.

Juice	Months of storage				$F_{Friedman}$*
	0	4	8	12	
A	81	97	98	94	3.00
B	95	111	117	97	4.91
C	108	121	128	123	2.73

* Critical $F_{Friedman}$ value = 7.82 (95% confidence).

4. Conclusions

The separation of the pulpy and low pulp fractions of orange juice and the application of different heating treatments to them is an interesting procedure to obtain different orange juices that keep the fresh taste. The treatment at 60 °C for 15 s of the resulting blend of the non-treated low pulp and the pasteurized pulpy fractions constitutes an alternative to produce high quality orange juice avoiding the constraints induced by handling operations under aseptic conditions. In addition, the procedures developed involve a considerable saving of thermal energy in the process of pasteurization.

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