Bacteriological and Shelf-Life Characteristics of Canned, Pasteurized Crab Cake Mix

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The bacteriological spoilage characteristics of a canned, pasteurized crab cake mix product stored at various temperatures were investigated. A large number of bacteria, both mesophilic and psychrophilic, survived the pasteurization process. *Bacillus* and *Micrococcus* were found to predominate when the product was stored at 30 C (86 F) and 18 C (64 F), whereas *Alcaligenes* predominated at 2 C (36 F). The product was found to be free of *Escherichia coli*. Bacterial counts, trimethylamine nitrogen, volatile reducing substances, and ammonia determinations were evaluated as indices of quality for the product. Close correlation was observed between bacterial counts, volatile reducing substance values, and organoleptic tests when the product was stored at 30 C (86 F). The shelf-life of the product was approximately 6 months at 2 C (36 F), 4 days at 18 C (64 F), and 27 hr at 30 C (86 F).

Canned, pasteurized crab cake mix is a relatively new product. There is little available information concerning its shelf-life and spoilage characteristics. The crab cake mix is commercially prepared by mixing crab meat with other ingredients, such as bread crumbs, eggs, mayonnaise, and other flavoring substances. The formula for the crab cake mix product is shown in Table 1. The product is packed at atmospheric pressure in 307 x 409 C enameled cans of 1 lb (454 g) net product capacity, pasteurized in a water bath at 85 to 87 C (185 to 190 F) for 110 min, and immediately cooled. The product is then kept at 0 to 5 C (32 to 41 F).

A problem may arise when the consumer removes the product from cold storage in a food store. In some instances, it may take several hours before the product is returned to a refrigerator in the consumer's home, or the product might inadvertently be left unrefrigerated overnight. Bacteriological changes occur during this period, some of which are undesirable from the standpoint of product quality, and perhaps safety. This problem is of interest to consumers and the industry.

Bacteriological spoilage of crab meat has been studied by Harris (15), Alford et al. (1), and Benarde (6). Pasteurization of crab meat in metal containers was first investigated by Tobin and McCleskey (27). They packed and pasteurized crab meat at 15 psi for 5, 10, and 15 min. They reported that slight discoloration of the surface of the pasteurized meat was observed.

Pasteurization of crab meat packed in cans in a water bath at the temperature below the boiling point of water was studied by Anzulovic and Reedy (4) and by Flynn and Tatro (14). These investigators found that pasteurization killed a large number of microorganisms including *Escherichia coli*, and that pasteurization prolonged the keeping quality of crab meat for a considerable time.

Much research has been done by employing trimethylamine nitrogen (TMA-N) as an index of decomposition of marine fish and shellfish. Spinelli et al. (25) studied the relation of bacterial counts and increase in TMA-N with sensory evaluation in vacuum-packed king crab meat. They found that samples with TMA-N content exceeding 1.0 mg/100 g received a poor rating. There appeared to be a fair correlation between increase in bacterial counts, TMA-N content, and a decrease in sensory scores. Farber (12) claimed that the content of TMA-N was not a sensitive, reliable, or reproducible index of fish spoilage. Either the increase in TMA-N occurred during the latter stage of spoilage, or there was a variation in levels between species, or no appreciable increase took place. Volatile reducing substances (VRS) content has been found to be a useful index of freshness of fresh and canned fishery products, as indicated by Farber (11),

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and Farber and Ferro (13). The content of VRS was reported to correlate closely with organoleptic judgments. Burnett (8) devised a colorimetric method which used ammonia as an index of decomposition in fresh and frozen crab meat. This method is based upon the color reaction between ammonia, thymol, and bromine. The author reported that ammonia content increased uniformly and rapidly with spoilage, and that ammonia could be detected before spoilage was detected organoleptically.

In crab cake mix there are ingredients of widely variable microbiological quality. The pasteurized product, therefore, is expected to have a variable spoilage pattern different from that of crab meat or fish. Generally accepted objective tests for ascertaining degree of spoilage of crab meat or fish may not be adequate for crab cake mix.

The principal objectives of this investigation were: (i) to determine the shelf-life of the product at various temperatures by studying the bacteriological spoilage pattern through bacterial counts and characterization of the more prevalent bacteria in the product; (ii) to evaluate some of the existing objective tests as indices of quality and degree of spoilage of the crab cake mix; and (iii) to determine the correlation between odor and bacterial count, TMA content, VRS value, and amount of ammonia present in the product.

**MATERIALS AND METHODS**

Rate of heat penetration in canned crab cake mix was determined under commercial conditions. The method described by Alstrand and Ecklund (2) was used. Six 307 × 499 cans of 1 lb (454 g) net product capacity were used for the determination. The molded bakelite thermocouples were first placed at the geometrical centers of the test cans before filling. Filling was done in such a way that the thermocouple tips were imbedded in the product with no air space around them. The canned product was pasteurized in a steam-heated water bath, previously heated to 85 °C (185 °F), for 110 min. Center can temperatures of the product were read before the beginning of the pasteurization process and every 10 min throughout the heating and during the cooling operation, until the temperature returned to 60 °C (140 °F). A Brown-potentiometer with automatic junction compensation was used to make temperature readings.

The general plate count techniques were those outlined in *Standard Methods for the Examination of Dairy Products* (3). Throughout this study, BBL standard plate count (SPC) agar was used for plate counts. For estimating bacterial population in fresh crab meat and crab cake mix, SPC agar was dissolved in artificial sea water (24).

Total plate counts of crab cake mix ingredients were determined. Samples of ingredients used for crab cake mix were obtained from a crab processing plant, placed aseptically in sterile bottles, and transported in ice to a laboratory where plate counts were made immediately. One gram of each of the ingredients was mixed with 9 ml of sterile water; the samples were further diluted and plated on SPC agar medium. All plates were prepared in triplicate and incubated at either 30 °C (86 °F), 18 °C (64 °F), or 2 °C (36 F) to determine the microflora growing in the samples at each of three temperatures. Plates were incubated at 30 °C (86 °F) for 48 hr, at 18 °C (64 °F) for 4 days, and at 2 °C (36 F) for 18 to 20 days before counting was made.

The bacteriological spoilage pattern of the product was studied at 30 °C (86 °F) to represent the upper extremes of room temperature storage, at 18 °C (64 °F) to include a temperature intermediate between room and refrigerated storage temperatures, and at 2 °C (36 F) to duplicate refrigerated storage temperature.

The more prevalent colonies of bacteria that grew on plates incubated at the three temperatures were isolated and differentiated according to genera. Routine characterization tests were done according to Bergey’s *Manual of Determinative Bacteriology* (7) and *Guide to the Identification of the Genera of Bacteria* (23). Flagella were stained by using the method of Leifson [E. Leifson, J. Bacteriol. 36:656 (Abstr.), 1938]. Oxidase activity was determined by the method of Kovacs (17).

Chemical analysis of the pasteurized crab cake mix for moisture, crude fat, protein, and ash were performed in accordance with Association of Official Agricultural Chemists techniques (16).

TMA-N content of pasteurized crab cake mix was determined colorimetrically as trimethylamine picate by the method of Dyer (10). A Spectronic 20 (Bausch & Lomb) colorimeter was used. TMA-N values were read as percentage of transmittance from the TMA-N standard curve.

VRS values were determined by the method of Lang et al. (18). The apparatus was slightly modified. It was found that simple test tubes (20 by 125 mm) could be used as sample vessels, since foaming was eliminated by using Antifoam A (Dow Chemical Co.). For each determination, 10 g of pasteurized crab cake mix, diluted 1:1 with distilled water, was weighed into an aeration vessel to which a few drops of the silicone antifoaming agent was added. Exactly 50 ml of 0.02 N KMnO₄ in 1.0 N NaOH solution was pipetted into a reaction flask. The determination of VRS values was carried out at room temperature. Room air was drawn into the VRS apparatus at a rate of 100 liter/hr by means of a vacuum pump. The sample was aerated for 40 min. An air blank was also determined by using 10

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**TABLE 1. Formula for 100 lb (45 kg) of crab cake mix**

| Ingredient         | Amount   |
|--------------------|----------|
| Crab meat (claw 70%, regular 30%) | 72 lb (32 kg) |
| Mayonnaise         | 10 lb (4.5 kg) |
| Bread crumbs       | 8.5 lb (3.8 kg) |
| Eggs               | 8.5 lb (3.8 kg) |
| Mustard            | 6 oz (170 g) |
| Pepper             | 6 oz (170 g) |
| Spice mix          | 2.5 oz (71 g) |
| Dry onion flakes   | 1.5 oz (42.5 g) |

The method is based upon the color reaction between ammonia, thymol, and bromine. The author reported that ammonia content increased uniformly and rapidly with spoilage, and that ammonia could be detected before spoilage was detected organoleptically.
ml of emulsified redistilled water, prepared by blending a few drops of the silicone antifoaming agent in 100 ml of redistilled water, in place of the sample.

At the end of the aeration period, the reaction flask was removed. A 25-ml amount of 6 N H$_2$SO$_4$ and 15 ml of 20% KI in 0.1% Na$_2$CO$_3$ were added. The liberated iodine was titrated with 0.025 N Na$_2$S$_2$O$_3$ in 0.2% Na$_2$CO$_3$ and 0.1% Na$_2$B$_4$O$_7$$.10$HzO solution. Toward the end of the titration, several drops of 1% soluble starch in saturated NaCl solution was added as an indicator. VRS value in the sample was calculated by the equation: VRS value = titration - sample titration X normality Na$_2$S$_2$O$_3$ X 1000/weight of sample in grams.

The VRS value determined as described above was expressed in microequivalent ($\mu$Eq) of KMnO$_4$ per gram of sample.

Ammonia content in the product was determined by the method of Burnett (8).

Odor of pasteurized crab cake mix, stored at room temperature for various periods of time, was evaluated by a panel of 20 persons. The panel was untrained but was made familiar with the product. A nine-point hedonic scale was used in rating the sample. Odor evaluations were made by using four replicates.

RESULTS

The rate of heat penetration in crab cake mix commercially packed in 307 X 409 C enameled cans is shown in Fig. 1. Approximately 100 min was required for the center can temperature to reach 82 C (180 F), the desired temperature for pasteurization. The center can temperature was maintained at 82 C (180 F) for approximately 10 min before the cans were cooled.

The pasteurization process at 85 to 88 C (185 to 190 F) for 110 min reduced the bacterial counts in the product by a factor of approximately 20, from 6.0 X 10$^4$ to approximately 3.3 X 10$^4$ per g.

Total plate counts of microorganisms in fresh crab meat and in other ingredients used for preparing crab cake mix are shown in Table 2. Bread crumbs showed fairly large counts of both mesophilic and psychrophilic bacteria, whereas pepper and spice mix contained rather large numbers of only mesophilic bacteria. A large number of both mesophilic and psychrophilic bacteria were found in raw crab cake mix.

Bacterial counts of the product stored at 30 C (86 F) are shown in Fig. 2. Bacterial counts from plates incubated at 30 C (86 F) and 18 C (64 F) increased with storage time, as might be expected. The increase in number of bacteria indicates

![Fig. 1. Heat penetration curve for crab cake mix in 307 X 409 cans with thermocouples at geometrical center of can.](image)

![Fig. 2. Bacterial counts for pasteurized crab cake mix stored at 30 C (86 F).](image)

| Ingredient          | Incubation temp (C)$^a$ |
|---------------------|-------------------------|
|                     | 2          | 18         | 30         |
| Regular meat        | 3.4 X 10$^4$ | 4.1 X 10$^4$ | 4.5 X 10$^4$ |
| Claw meat           | 1.5 X 10$^4$ | 3.1 X 10$^4$ | 4.1 X 10$^4$ |
| Pepper              | <300       | 1.5 X 10$^8$ | 2.0 X 10$^8$ |
| Spice mix           | <300       | 1.2 X 10$^6$ | 0.9 X 10$^6$ |
| Bread crumbs        | 2.6 X 10$^4$ | 2.4 X 10$^6$ | 2.0 X 10$^6$ |
| Dry onion flakes    | <300       | 2.1 X 10$^4$ | 2.9 X 10$^4$ |
| Mayonnaise          | <300       | <300       | <300       |
| Mustard             | <300       | <300       | <300       |
| Raw crab cake mix   | 2.4 X 10$^4$ | 3.4 X 10$^6$ | 6.0 X 10$^4$ |

$^a$ Results expressed as microorganisms per gram.
typical growth curves which clearly show lag, log, and stationary phases. The product contained approximately $2 \times 10^4$ bacteria per g at zero storage time. The counts increased significantly after approximately 10 to 12 hr of storage time at 30°C. This can be explained by the fact that the rate of heat penetration in the product is slow. After 24 hr, the product showed a sign of slight spoilage, as observed by odor. Bacterial counts showed approximately $10^8$ organisms per g. This indicates that the shelf-life of the product stored at 30°C (86°F) is in the neighborhood of 24 hr. When plates were incubated at 2°C (36°F), the number of bacteria, presumably psychrophiles, increased slightly up to approximately 12 hr of storage time at 30°C (86°F) and declined significantly thereafter. The increase in number of bacteria at the beginning indicated that the temperatures in the can of crab cake mix were suitable for psychrophilic bacteria to grow up to approximately 12 hr of storage time. The rapid decrease in the number of bacteria after 12 hr of storage is postulated to be due to the genetic behavior of the psychrophiles in the product. These psychrophiles presumably came from a cold marine environment in the Chesapeake Bay area. When exposed to high storage temperatures, they tended to die.

Bacterial counts in the product stored at 18°C (64°F) for various lengths of time are shown in Fig. 3. When plates were incubated at each of the three incubation temperatures, both mesophilic and psychrophilic bacteria appeared to grow well. This is due to the fact that the storage temperature of 18°C (64°F) is within the growing ranges of both types of bacteria. However, the mesophiles seemed to grow better than the psychrophiles after 2 days of storage. After 4 days of storage at 18°C (64°F), an off odor was noted in the product. Bacterial count at this time showed approximately $7 \times 10^3$ organisms per g.

Bacterial counts of the product stored at 2°C (36°F) were made at 1-month intervals for 6 months (Fig. 4). It was observed that the counts of both mesophilic and psychrophilic bacteria were fairly low, even after 6 months of storage time. A slight off odor was observed at the end of that period, but the product was still considered acceptable. The shelf-life of the product at 2°C (36°F) was considered to be 6 months.

The next step in the bacteriological examination of pasteurized crab cake mix was to characterize bacteria isolated from the product stored at different temperatures. Attempts were made to characterize the more prevalent bacteria in the product stored at the three temperatures, namely, 30°C (86°F), 18°C (64°F), and 2°C (36°F). Three genera of bacteria were found to be prominent in pasteurized crab cake mix stored at the three temperatures (Table 3). They were *Bacillus*, *Micrococcus*, and *Alcaligenes*. *Bacillus* was characterized mainly according to its ability to survive the heat treatment of 65°C (149°F) for 30 min, and as catalase-positive, gram-positive, aerobic, spore-forming bacilli. *Micrococcus* was characterized mainly as gram-positive cocci in clumps, for producing acid from glucose oxidatively, and for having the ability to survive the heat treatment. *Alcaligenes* was characterized as gram-negative and oxidase-positive, for being motile with peritrichous flagella, for its inability to utilize carbohydrates, and for itsropy colonies.

Attempts were made to detect *E. coli* in pas-
Table 3. Characterization of the more prevalent bacteria isolated from commercially prepared, pasteurized crab cake mix

| Characteristics                  | Bacillus    | Micrococcus | Alcaligenes |
|----------------------------------|-------------|-------------|-------------|
| Morphology                       | Straight rod| Coccii, in clump | Straight rod |
| Growth in air                    | +           | +           | +           |
| Optimal growth temp (C)          | 30          | 30          | 18          |
| Gram reaction                    | G+          | G+          | G-          |
| Glucose reaction                 | A           | A           | -           |
| Acid from glucose                | +           | +           | -           |
| Oxidative                        | -           | -           | -           |
| Fermentative                     | -           | -           | -           |
| Sucrose reaction                 | A           | A           | -           |
| Lactose reaction                 | -           | -           | -           |
| Litmus milk reaction             | Proteolysis | Proteolysis | Slight proteolysis |
| Catalase reaction                | +           | +           | +           |
| Survived heat treatment (65 C for 30 min) | +           | NA          | NA          |
| Spore stain                      | +           | NA          | NA          |
| Motility                         | +           | NA          | +           |
| Flagella stain                   | NA          | NA          | Peritrichous+|
| Oxidase activity                 | NA          | NA          | +           |
| Gelatin reaction                 | Liquefaction| -           | NA          |

a +, Positive reaction; -, negative reaction; G+, gram-positive; G-, gram-negative; A, acid production; NA, test does not apply.

Table 4. pH values of pasteurized crab cake mix stored at 30 C (86 F), 18 C (64 F), and 2 C (36 F)

| Storage time (hr) | pH | Storage time (days) | pH | Storage time (days) | pH |
|-------------------|----|---------------------|----|---------------------|----|
| 30 C (86 F) storage |    | 18 C (64 F) storage |    | 2 C (36 F) storage |    |
| 0                 | 7.22 | 0                  | 7.22 | 0                | 7.22 |
| 4                 | 7.10 | 0.5                | 7.12 | 4                | 7.20 |
| 8                 | 7.18 | 1                  | 7.10 | 8                | 7.10 |
| 12                | 7.12 | 2                  | 7.17 | 12               | 7.20 |
| 24                | 7.10 | 3                  | 7.10 | 16               | 5.17 |
| 48                | 6.50 | 4                  | 6.90 | 20               | 7.10 |

Mean values from three determinations.

The pH values of samples of pasteurized crab cake mix stored at 30 C (86 F), 18 C (64 F), and 2 C (36 F) are shown in Table 4. Determinations of pH in the product at each storage temperature indicate that, although pH of the samples in advanced stages of spoilage showed a lower value than that of fresh samples, the samples in between inhibited a nonuniform decrease of pH. No significant difference was found in the pH of samples in borderline stages of spoilage when compared with the pH of relatively fresh samples. Therefore, pH is not a reliable index of decomposition for pasteurized crab cake mix.

Proximate composition data for pasteurized crab cake mix are presented in Table 5. The quantity of crude fat in the product is relatively high because a rather high proportion of mayonnaise is included in the formula.

TMA-N content of pasteurized crab cake mix stored at 30 C (86 F) for various periods of time is shown in Fig. 5. It is evident that TMA-N content increased significantly after approximately 24 hr of storage, or when the product was approaching an advanced stage of spoilage. No significant difference was found in TMA-N content of the product stored at zero time, 6, 12, and 24 hr. These results show that TMA-N is significant.

Table 5. Proximate composition of pasteurized crab cake mix

| Determination | Moisture | Protein (N X 6.25) | Fat | Ash | Carbohydrates (by difference) |
|---------------|---------|--------------------|-----|-----|-------------------------------|
|               | %       | %                  | %   | %   | %                             |
| \( \bar{x} \) | 68.37   | 14.58              | 10.04 | 2.95 | 4.06                          |
| S               | 0.22    | 0.61               | 0.24 | 0.08 |                               |

a Mean value from 10 determinations.

b Standard deviation.
The average odor scores for the product stored at 30 °C (86 °F) are also presented in Fig. 5. There was no significant difference between zero exposure and exposure to 30 °C (86 °F) for 6 hr. The product exposed for 12 hr at 30 °C (86 °F) showed no significant difference from that with zero exposure. The product exposed to 30 °C for 24 hr was significantly different from any of the product exposed for shorter periods of time. This product, however, was also significantly different from the product exposed to 30 °C for 30 hr which was judged unacceptable. This result indicates that the quality of pasteurized crab cake mix stored for 24 hr at room temperature was approaching the borderline stage of spoilage, although it was still considered acceptable. On the contrary, the product exposed to 30 °C for 30 hr at room temperature was considered unacceptable. The borderline stage of spoilage, therefore, appeared to occur between 24 and 30 hr of storage time and was extrapolated to be approximately 27 hr at 30 °C (86 °F).

A recovery test was employed to determine the efficiency of the colorimetric method used for detecting amount of ammonia (NH₃) in the product. It was found that the determination of NH₃ in the product was not reliable as a test for product quality, as the results were not reproducible. It was concluded that other substances in the product were probably extracted along with NH₃ and interfered with the colorimetric determination.

On the basis of the results given above, shelf-life of the product was approximately 27 hr at 30 °C (86 °F), 4 days at 18 °C (64 °F), and 6 months at 2 °C (36 °F).

**DISCUSSION**

Bacterial counts have been used by many investigators to follow the deterioration of fish and shellfish. In this study, the product immediately after pasteurization had total plate counts of approximately $3.5 \times 10^4$ per g. When the product was stored at 30 °C (86 °F), the count increased to approximately $10^5$ at the time of spoilage. The bacterial counts of the product stored at 30 °C appeared to correlate with the degree of acceptability as indicated by odor. The bacterial counts, therefore, appeared to be a good index of spoilage of the product when stored at 30 °C, as shown in Fig. 5. When the product was stored under refrigeration, the increase in bacterial counts showed no correlation with degree of spoilage. Figure 4 shows that approximately $10^7$ bacteria per g were detected in the product held refrigerated [2 °C (36 °F)] for 6 months, when plates were incubated at 2 °C. The count was typical for spoiled product exposed to room temperature, yet the product was still considered acceptable. These results indicate that the type of bacteria in the product is also important as an index of spoilage.
product and the incubation temperature of the plates appeared to be significant when using bacterial count as an index of spoilage. A disadvantage of using plate count as an index of spoilage is the length of time involved in incubation.

Bacteriological spoilage of the product is considerably retarded if the product is held refrigerated. *Bacillus* and *Micrococcus* showed optimal growth at 30 C (86 F). These bacteria are then expected to contribute very little to spoilage of the product at refrigerated storage temperatures. *Alcaligenes*, although showing optimal growth at 18 C (64 F), can grow well at refrigerated storage temperatures.

Work done by Tobin et al. (26), Reay and Shewan (20), Campbell and Williams (9), and others indicated that the *Pseudomonas-Achromobacter* group of bacteria contributes significantly to the spoilage of most fishery products held at refrigerated storage temperatures. However, this group of bacteria was not found in pasteurized crab cake mix, probably as the consequence of severe heat treatment during pasteurization. This result agrees with that of Macaulay et al. (19), who reported that *Alcaligenes* had relatively higher heat resistance that the pseudomonads. No reports have been found in the literature indicating that *Alcaligenes* contributes significantly to the spoilage of fishery products. In this study, *Alcaligenes* was found to be abundant in pasteurized crab cake mix, but it did not appear to contribute significantly to the spoilage of the product at refrigerated storage temperatures [2 C (36 F)]. Probably for this reason, the product was considered acceptable after a 6-month storage period.

The TMA-N content in pasteurized crab cake mix was found not to be a sensitive index of spoilage of the product. The TMA-N content appeared to increase during the latter stages of spoilage. Early changes in quality of the product could not be detected by the TMA-N method.

 Determination of the VRS value of pasteurized crab cake mix can be carried out in less than 1 hr. The VRS values of the product were found to increase with length of the storage time. A close correlation was observed between the VRS values and organoleptic judgments. More significantly, quality changes in the product could be detected chemically by VRS values during the early stages of decomposition. VRS values, therefore, appear to provide a sensitive and reliable measure of the quality of pasteurized crab cake mix.

The determination of ammonia in the crab cake mix product by a colorimetric method was not found reliable as an index of quality.

Canned, pasteurized crab cake mix is prepared by mixing crab meat with other ingredients, some of which are heavily contaminated with microorganisms, such as the spices. The product is pasteurized in a water bath at 85 to 88 C (185 to 190 F) for 110 min. It is possible that *Clostridium botulinum* types A and B, which are common soil organisms, and types E and F, which have been isolated from fish and shellfish, may be present in the product and survive the pasteurization process. Moreover, the condition of the product in the can is favorable for growth of *C. botulinum*. As the product is a semisolid mass, anaerobic conditions could exist in it. *C. botulinum* type E is known to grow and produce toxin after 1 month of incubation at 38 F as reported by Schmidt et al. (21).

To avoid the possibility of microbial growth and toxin production, the product would have to be stored at temperatures below 38 F. To prevent physicochemical changes caused by freezing, the storage temperature should be higher than the freezing temperature of any of the product components. Food storage temperatures in the range of about 28 to 38 F are rarely found in retail stores and homes. This presents a serious problem in marketing products like the one discussed here, and other similar meat and seafood products packed in hermetically sealed containers but not given a heat treatment that results in commercial sterilization.

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