



Nutritional Quality and Shelf Life of Processed Tomato Juice and Paste Using *Rosmarinus officinalis* Essential Oil Combined with Low Heat Treatment in Challenged Conditions with *Bacillus cereus* Spores

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CIS, SLSK, GMN and JJEN designed the study. Author CIS wrote the protocol, managed the analyses of the study and literature searches with author SLSK. Author SLSK performed the statistical analysis with author CIS. Authors CIS, SLSK, GMN and JJEN wrote the first draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This experiment was done to investigate the effect of adding *Rosmarinus officinalis* essential oil (REO) in tomato juice and paste during processing in condition of reduced thermal treatment on nutritional qualities and evaluate their postharvest shelf life under *Bacillus cereus* spore contamination.

Place and Duration of Study: Laboratory of Microbiology, University of Yaoundé I, Cameroon, from January 2017 to June 2018.

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Methodology: Total polyphenol content was determined using the Folin Ciocalteu coloric method; the Titratable acidity and vitamin C content were determined by titration with indicator method. The effect of level of *Bacillus cereus* contamination, time of thermal treatment and concentration of REO was assessed using a experimental CCD plan. The shelf life was estimated using the storage efficacy of the product on a period of time, and calculated based on the percentage of spoiled products. Data were analysed using XLSLTAT and STATISTICA packages for non-linear regression analyses.

Results: Only 10 min pasteurization at 95°C in the presence of 100ppm of ROE was necessary to totally deactivate 10^4 *Bacillus cereus* spores/g in tomato juice while for tomato paste the same concentration of ROE needed 20 min pasteurization at 95°C to totally deactivate up to 10^8 *Bacillus cereus* spores/g. Total polyphenols, Titratable acidity and Vitamin C content were affected under this combined processing technique. The storage efficacy of tomato products and hence the extended shelf life was as a result of combined effects between cooking time and microbial load percent reduction that depended on the spore load concentration.

Conclusion: Using *Rosmarinus officinalis* essential oil can help reduce the thermal impact during tomato juice and paste processing even in condition of high contamination of spores of *Bacillus cereus*. Moreover in this processing condition, nutritional compounds are not significantly impacted.

Keywords: *B. cereus*; postharvest quality; rosemary essential oil; shelf life; tomato products.

1. INTRODUCTION

Attention to the concept of postharvest food loss reduction as a significant means to increase food availability was drawn by the World Food Conference held in Rome in 1974 [1] Postharvest losses are much higher for perishable fresh fruits and vegetables than for cereals and other field crops. Once harvested, fruits and vegetables have a limited postharvest life. It has been reported that in developing countries, up to 30% of the food produced cannot be used due to the spoilage by the actions of microorganisms, rodents, or insects according to UNDP reports, food wastes represent 33% of food production. Storage and processing technologies have been utilised for centuries to transform these perishable fruits and vegetables into safe, delicious and stable products. Washing, peeling and blanching steps prior to processing are responsible for some loss of water-soluble nutrients. Depending on how processing is carried out, it may result in changes in colour, texture, flavour and nutritional quality [2]. Food processing techniques, among other goals, reduce the number of microorganisms present or inhibit their growth in food [3,4], although there is a risk that processing steps may also contaminate the final product [5]. Thermal treatment (pasteurisation and sterilisation) is the most common and widely employed method for the inactivation of microorganisms and enzymes in the food industry [6]. Tomato (*Lycopersicon esculentum* Mill.) is a plant fruit of great economic importance in most of the developing countries because of its availability in different

daily meals. The demand for tomato processing typically arises from a need to preserve the product for cooking purposes. Tomatoes are processed into many different tomato-based products such as sauce, soup, paste and juice [7]. Tomatoes and tomato-based products provide a wide variety of nutrients and many health-related benefits to the body [8]; these health benefits are related to the ingestion of bioactive components such as essential vitamins, minerals and polyphenolic compounds. Microbial contamination has been always considered to be due to bacteria, moulds or yeasts, which can survive in the processed products. These microorganisms may promote the deterioration of food products by degrading some of their compounds (such as carbohydrates, protein and vitamins), producing undesirable odour and off-flavour, coloration, pH and texture changes [9]. Bacterial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety of these foods [10]. Sporulating forms have a distinct role in food deterioration; this, due to the high thermal resistance of the spores, which in some species are still viable after the high temperature treatments associated with the pasteurization process. *Bacillus* and *Clostridium* are the few genera that can grow in such substrate. *Bacillus cereus* is the most important contaminant of the spore forming microorganisms because its spores are ubiquitous and can therefore survive cooking and dry storage [11,12]. Being a soil resident, *B. cereus* can spread easily into many types of foods such as plants, eggs, meat, fish, milk, and

dairy products, and is known for causing 25% of food-borne intoxications [13]. Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in foods while maintaining quality, freshness, and safety [14,15]. The use of essential oils is becoming popular to increase the shelf life of food products, since consumers are more conscious about the health problems caused by several synthetic preservatives [16,17,18,19]. Spices and herbal essential oils have been used by the food industry as natural agents for extending the shelf life of foods. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property [15]. A variety of plant based antimicrobials is used for reducing or eliminating pathogenic bacteria and increasing the overall quality of food products. Among several essential oils that may be useful as antimicrobial agents,

rosemary (*Rosmarinus officinalis*) has several applications especially in the food processing and preserving because of its natural antioxidant and antimicrobial properties [10,20,21]. This study aimed to investigate if the use of rosemary essential oil (*Rosmarinus officinalis*) could permit to reduce thermal treatment time and nutrient loss during the production of tomatoes paste and juice, while maintaining food safety in situation of *Bacillus cereus* contamination.

2. MATERIALS AND METHODS

2.1 Sample Preparations

Tomato fruits (*Rio Grande cv.*) were purchased from a local supplier at a commercial stage of ripeness. Fruits were sorted and washed with tap-water to remove dirt and soil, before processing into juice and paste (Fig. 1).

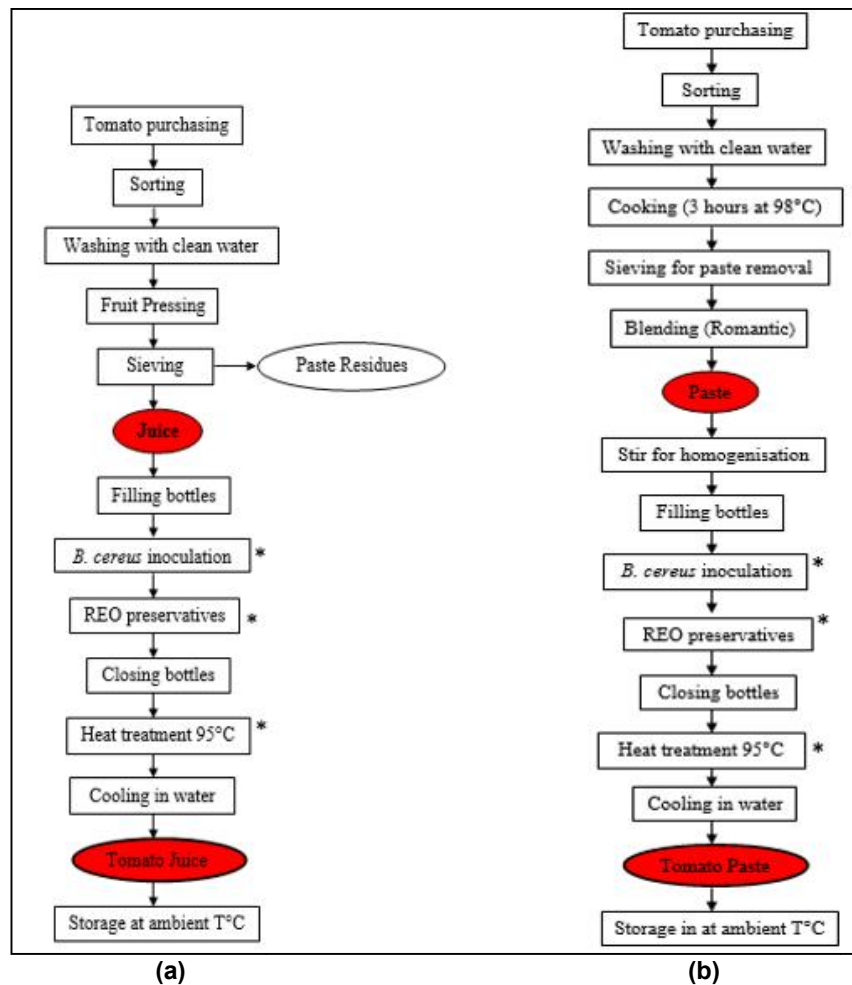


Fig. 1. Flow chart of processing tomato juice (a) and tomato paste (b)

* According to the experimental plan

2.2 Preparation of *Bacillus cereus* Spores

To induce sporulation of vegetative cells of *B. cereus* (ATCC 11966), the procedures described by Etoa, et al. [22] were used. Briefly, spores stored on nutrient agar slants were cultured in nutrient broth and incubated at 37°C for 24 hours. Later, 10³ cells/ml of the inoculum were sporulated on nutrient agar supplemented with salts (0.5 g of disodium phosphate, 0.1 g of calcium chloride, 0.04 g of manganese sulphate), then incubated for 7 days at 37°C. After these days, spores were harvested by flooding the plates with sterile distilled water. Purification of spores was performed by several centrifugations at 4000 g/15 min at 4°C and stored at 4°C for 1 month before use.

2.3 Experimental Design and Treatments

Three treatment factors namely *Bacillus cereus* concentration deliberately inoculated (F₁), Cooking time (F₂) and Rosemary essential oil (REO) concentration (F₃) were considered at 5 levels (-2, -1, 0, 1, 2) each in a central composite experimental design (CCD) reinforced at the edges as indicated in Tables 1-2.

2.4 Enumeration of Residual Spores in Juice and Paste

Residual spores or vegetative cells of *Bacillus cereus* in juice or paste treated according to the reinforced experimental CCD were determined using a three repetition most probable number protocol with dilutions ranging from 1 to 10⁻⁵ dilutions. After incubation of the diluted samples at 37°C for 24 hours, observations on the number of tubes per repetitions presenting *B. cereus* growth were recorded. The generated code was used to calculate the MPN cells/g or ml using a MPN Excel add-in provided by Jarvis, et al. [23].

2.5 Physicochemical Analyses

2.5.1 Total polyphenols

Total polyphenols content were determined using a modified Folin-Ciocalteu colorimetric method [24,25]. After incubation at room temperature for 90 minutes, the absorbance was read at 760 nm using a UV-VIS Spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg of Gallic acid equivalents (mg GAE) per ml of tomato juice or per mg of tomato paste. The correlation coefficient for the calibration curve was 0.9958.

Table 1. Corresponding values for tomato juice in a Central Composite Design (CCD) plan

Coded values in the CCD				Corresponding real values			
Condition	F ₁	F ₂	F ₃	Condition	<i>B. cereus</i> (log)	Time (min)	REO (ppm)
1	-1	-1	-1	1	2	5	50
2	-1	1	1	2	2	20	150
3	1	-1	1	3	6	5	150
4	1	1	-1	4	6	20	50
5	0	0	0	5	4	10	100
6	-1	-1	1	6	2	5	150
7	-1	1	-1	7	2	20	200
8	1	-1	-1	8	6	5	50
9	1	1	1	9	6	20	150
10	0	0	0	10	4	10	100
11	-2	0	0	11	0.1	10	100
12	2	0	0	12	8	10	100
13	0	-2	0	13	4	0	100
14	0	2	0	14	4	30	100
15	0	0	-2	15	4	10	0
16	0	0	2	16	4	10	200
17	0	0	0	17	4	10	100
18	2	-2	2	18	8	0	200
19	2	2	2	19	8	30	200
20	-2	-2	-2	20	0.1	0	0

F₁: *Bacillus cereus* spores (log cell/mL); F₂: Cooking time (minutes); F₃: Rosemary essential oil (ppm), v/v

Table 2. Corresponding values for tomato paste in a Central Composite Design (CCD) plan

Coded values in the CCD				Corresponding real values			
Condition	F ₁	F ₂	F ₃	Condition	<i>B. cereus</i> (log)	Time (min)	REO (ppm)
1	-1	-1	-1	1	2	10	50
2	-1	1	1	2	2	30	150
3	1	-1	1	3	6	10	150
4	1	1	-1	4	6	30	50
5	0	0	0	5	4	20	100
6	-1	-1	1	6	2	10	150
7	-1	1	-1	7	2	30	200
8	1	-1	-1	8	6	10	50
9	1	1	1	9	6	30	150
10	0	0	0	10	4	20	100
11	-2	0	0	11	0.1	20	100
12	2	0	0	12	8	20	100
13	0	-2	0	13	4	0	100
14	0	2	0	14	4	40	100
15	0	0	-2	15	4	20	0
16	0	0	2	16	4	20	200
17	0	0	0	17	4	20	100
18	2	-2	2	18	8	0	200
19	2	2	2	19	8	40	200
20	-2	-2	-2	20	0.1	0	0

F₁: *Bacillus cereus* spores (log cell/mL); F₂: Cooking time (minutes); F₃: Rosemary essential oil (ppm), v/v

2.5.2 pH and titratable acidity

The pH values of tomato juice and paste were measured by using electronic pH meter (Hanna) [26]. Titratable acidity (TA) was obtained by titrating 5 ml of tomato extracts with an alkaline solution (0.1 N NaOH) using phenolphthalein indicator up to pH 8.1. The appearance of light pink colour was marked as the end point. Results were expressed as grams of citric acid per 100 g of tomato products [27,28,29].

2.5.3 Vitamin C concentration

Vitamin C in tomato juice and paste was estimated by the indicator method. The vitamin C contents of fruit juices were reported as mg/100 ml [26,30].

2.5.4 Shelf life of tomato juice and paste

In order to analyse the data for shelf life recorded for one year, a storage efficacy of the process was calculated using the following formula developed during this study:

$$\text{Shelf life} = \frac{\sum(\text{number of good samples on Day } i \times \text{Day } i)}{(\text{Maximum sample repetition} \times \sum \text{Day } i)}$$

The value ranges from 0 to 1: 0 being a condition where all repetitions got spoiled on the first day of storage, and 1, a process condition that have

assured all sample repetitions stable until 366 days (1 year).

3. RESULTS

3.1 Microbial Deactivation

Deliberately inoculated tomato juice and paste were produced as indicated in Fig. 1. After appertisation, the percentage of microbial reduction was assessed before the beginning of storage at ambient temperature. These results are presented in Tables 3-4.

The results (Table 3) were analysed using a non-linear regression in order to assess the relations between factors and the percentage reduction. The percentage reduction of *B. cereus* in juice is given in the below equation with R²= 0.32 and SE= 1351

$$\% \text{ Reduction} = 93.5 + 0.1542*[B. \text{ cereus}]*[\text{Time}] - 0.0158*[B. \text{ cereus}]*[\text{REO}] \quad (\text{Equation 1})$$

It indicates that interactions between treatments (*Bacillus cereus* concentration and cooking time; *Bacillus cereus* concentration and REO concentration) were responsible for the spore reduction. Maximum reduction (100%) of *B. cereus* was observed in J5, J10, J11, J13, J14, J17 J19 and J20 where there was mostly a concentration of 10⁴ *B. cereus*/ml, cooking time

(10 minutes) and 100 ppm of REO. It can be observed that the natural spore content of the product estimated to 0.1 Log ufc/ml (treatment J20) could not be detected in the product before storage.

In Table 4, the combination of a time of 20 minutes and REO of 100 ppm seems the best, since 100% reduction were noted in P10 and P12. The increase in essential oil over 100 ppm while maintaining thermal treatment time to 20 minutes did not provide a better bacterial reduction. In order to assess the relation between factors and the percentage reduction, a non-linear regression was performed and the following equation obtained:

$$\% \text{ Reduction} = 92.422 + 0.179*[\text{REO}]*[B. \text{ cereus}] - 0.428*[B. \text{ cereus}]*[\text{Time}] \quad (\text{Equation 2})$$

$$[R^2 = 0.35 \text{ and SE} = 62.9]$$

It can be observed that the combination of *B. cereus* concentration with time and REO concentration affected significantly the percentage reduction of the strain.

3.2 Total Polyphenols

Significant differences among treatments with regards to the concentration of total polyphenols (µg/ml juice and µg/mg paste) were observed (Figs. 2 and 3), and different groups were formed statistically ($p < 0.05$). In Fig. 2, three different groups were formed statistically ($p < 0.05$), and the higher concentration was recorded in J2 (167.22 µg/ml juice), whereas the lower concentration was observed in J9 (40 µg/ml juice). In Fig. 3, significant differences ($p < 0.05$)

were observed in most treatments and 2 groups were formed from the mean comparison. The most high concentrations of total polyphenols (µg/mg paste) was recorded in P1 (220 µg/mg paste) and the lower concentration in P5 (85.56 µg/mg paste).

3.3 pH and Titratable Acidity

The ph of tomato juice and tomato paste were 4.6 ± 0.3 and 4.7 ± 0.4 respectively. Results on the concentration of titratable acidity in tomato products (Figs. 4 and 5) were quite constant during the different treatments although a significant difference was observed between the highest and lowest values. In tomato juice (Fig. 4), the highest and lowest values were found respectively in J18 (29.44 g/100 ml juice); and in J11 (16.32 g/100 ml juice) while In tomato paste (Fig. 5), they were respectively found in P1 (35.84 g/100 g paste) and in P12 (28.8 g/100 g paste).

3.4 Vitamin C Concentration

Statistical analyses have indicated significant differences among treatments with regards to the vitamin C concentration level (Figs. 6 and 7). In tomato juice (Fig. 6), four different groups resulted in the mean comparison, though independently of the factors. The high concentration was found in J1 (20.43 mg/ml) and the low concentration in J20 (14.40 mg/ml). The concentration of vitamin C in tomato paste (Fig. 7) has been reduced compared to tomato juice; and significant differences were found among treatments with the highest concentration being in P12 (2.93 mg/mg) and the lowest P20 (0.47 mg/mg).

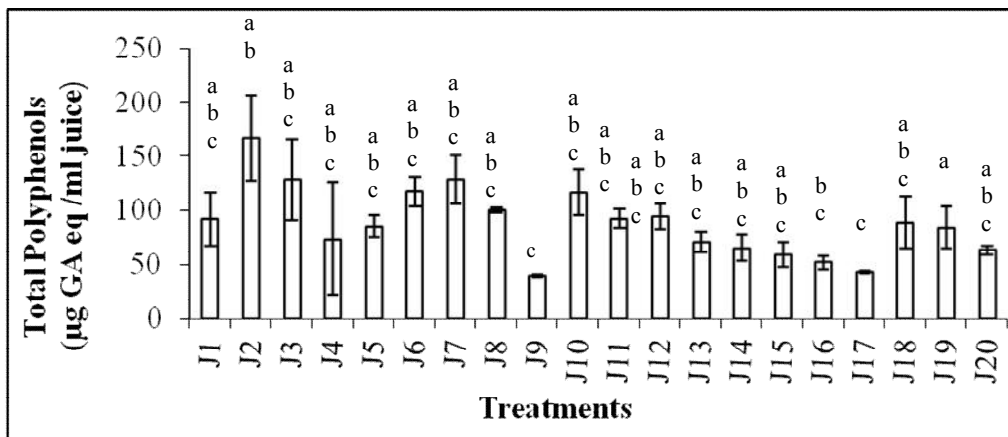


Fig. 2. Levels of total polyphenols in tomato juice as affected by different treatments J1, J2, J3... J20: Labels for Juice different treatment conditions

Table 3. Microbial load reduction in tomato juice, storage efficacy and shelf life obtained after treatment at 95°C for varied time of appertisation, concentrations in *B. cereus* and REO

Juice Treatments	<i>B. cereus</i> (log CFU/g)	Time (min)	REO (ppm)	MPN/ml	% reduction	Juice storage efficacy	Shelf life (months)
J1	2	5	50	38.01	61.99	1.00	12
J2	2	20	150	3.00	97.00	1.00	12
J3	6	5	150	18.00	99.99	1.00	12
J4	6	20	50	9.16	99.99	0.79	9.6
J5	4	10	100	0.00	100.00	1.00	12
J6	2	5	150	3.57	96.43	1.00	12
J7	2	20	200	3.00	97.00	0.79	9.5
J8	6	5	50	21.00	99.99	0.93	11.2
J9	6	20	150	146.63	99.99	0.75	9
J10	4	10	100	0.00	100.00	0.75	9
J11	0.1	10	100	0.00	100.00	0.54	6.5
J12	8	10	100	920.00	99.99	0.75	9
J13	4	0	100	0.00	100.00	0.50	6
J14	4	30	100	0.00	100.00	0.54	6.5
J15	4	10	0	3.00	99.97	0.75	9
J16	4	10	200	3.00	99.97	0.77	9.2
J17	4	10	100	0.00	100.00	0.79	9.5
J18	8	0	200	43653877.62	56.35	0.79	9.5
J19	8	30	200	198.25	100.00	0.79	9.5
J20	0.1	0	0	0.00	100.00	0.54	6.5

J1, J2, J3... J20: Labels for Juice treatments

Table 4. Microbial load reduction in tomato paste, storage efficacy and shelf life obtained after treatment at 95°C for varied time of appertisation, concentrations in *B. cereus* and REO

Paste Treatments	<i>B. cereus</i> (log CFU/g)	Time (min)	REO (ppm)	MPN/ml	% reduction	Paste storage efficacy	Shelf life (months)
P1	2	10	50	1.0025	99.95	0.84	10.1
P2	2	30	150	1.0025	99.95	0.79	9.5
P3	6	10	150	1.0025	99.98	0.50	6
P4	6	30	50	92	67.27	0.54	6.5
P5	4	20	100	1.0025	99.97	1.00	12
P6	2	10	150	1.0025	99.95	1.00	12
P7	2	30	200	1.0025	99.95	0.59	7.1
P8	6	10	50	21	77.96	0.59	7.1
P9	6	30	150	9.17704	83.95	0.54	6.5
P10	4	20	100	0.0000	100.00	1.00	12
P11	0.1	20	100	1.0025	98.92	0.75	9
P12	8	20	100	930000	100.00	0.79	9.5
P13	4	0	100	9300	0.79	0.17	2
P14	4	40	100	1.0025	99.97	0.54	6.5
P15	4	20	0	1.0025	99.97	1.00	12
P16	4	20	200	1.0025	99.97	1.00	12
P17	4	20	100	1.0025	99.97	0.84	10.1
P18	8	0	200	2.4x 10 ⁺⁰⁸	0.10	0.03	0.4
P19	8	40	200	42	79.71	0.66	7.9
P20	0.1	0	0	23	0.00*	0.84	10.1

* affected because an increase in cells was observed; P1, P2, P3... P20: Labels for Paste treatments

3.5 Shelf Life of Tomato Juice and Paste

The conservation length based on microbial macroscopic observations for both tomato juice (Table 3) and paste (Table 4) was affected by *B. cereus* contamination, REO concentration and time of appertisation. The best shelf life obtained in this experiment was one year.

In tomato juice (Table 3), treatments that had initial spore load of 2 log concentration of *B. cereus* combined with different REO concentrations and appertisation had a prolonged shelf life. However, the interaction between REO and thermal treatments is not very straight forward. In fact, when using 200 ppm of REO, the same level of inactivation was

observed without treatment (J18) and after 20 minutes (J4) at 95°C.

In tomato paste (Table 4), treatments with initial spore load of 4 *B. cereus* log concentration and pasteurization time of 20 minutes combined with different REO concentrations had a shelf life of at least 1 year. Moreover, when the inoculated load was 2 log, a treatment time of 10 minutes and REO of 50 ppm could assure a 1 year of shelf life. It can also be observed that tomato paste with 20 minutes appertisation and no REO concentration (P15) demonstrated also a 1 year of storage level. Time of treatment higher than 30 minutes in combination of REO were not in favour of the storage efficacy. The use of REO alone gave lower storage times inversely proportional to the microbial initial load.

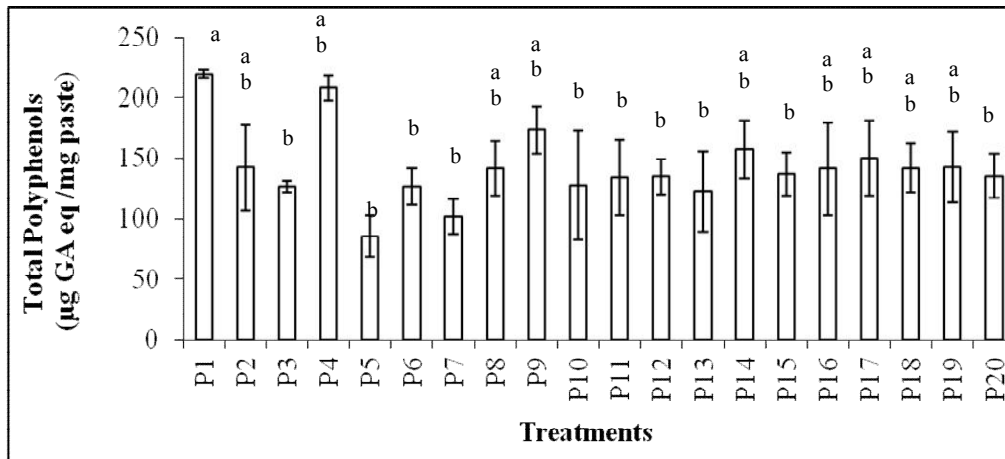


Fig. 3. Levels of total polyphenols in tomato paste as affected by different treatments
P1, P2, P3 ... P20: Labels for Paste different treatment conditions

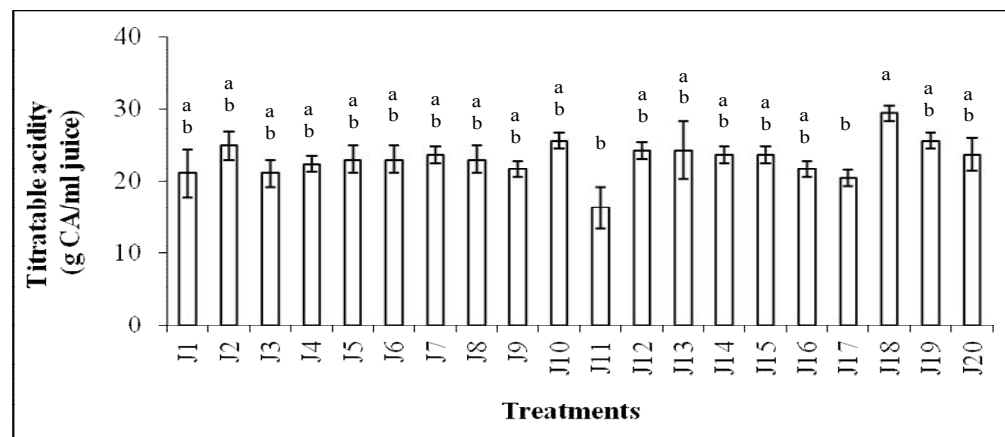


Fig. 4. Levels of Titratable acidity in tomato juice as affected by different treatments
J1, J2, J3... J20: Labels for Juice different treatment conditions

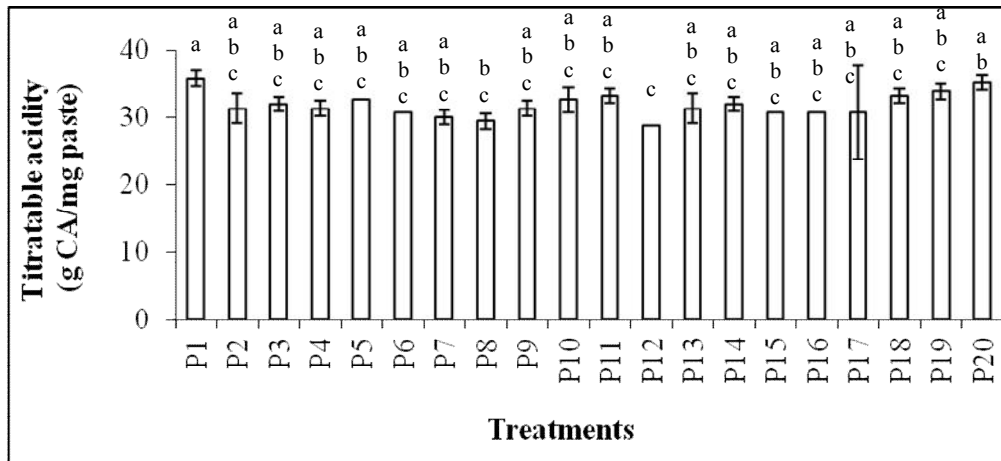


Fig. 5. Levels of Titratable acidity in tomato paste as affected by different treatments
P1, P2, P3 ... P20: Labels for Paste different treatment conditions

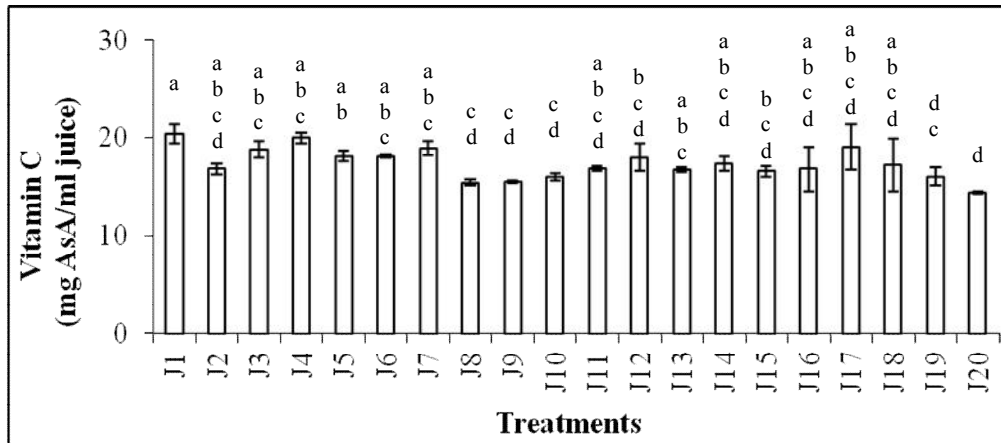


Fig. 6. Levels of Vitamin C in tomato juice as affected by different treatments
J1, J2, J3... J20: Labels for Juice different treatment conditions

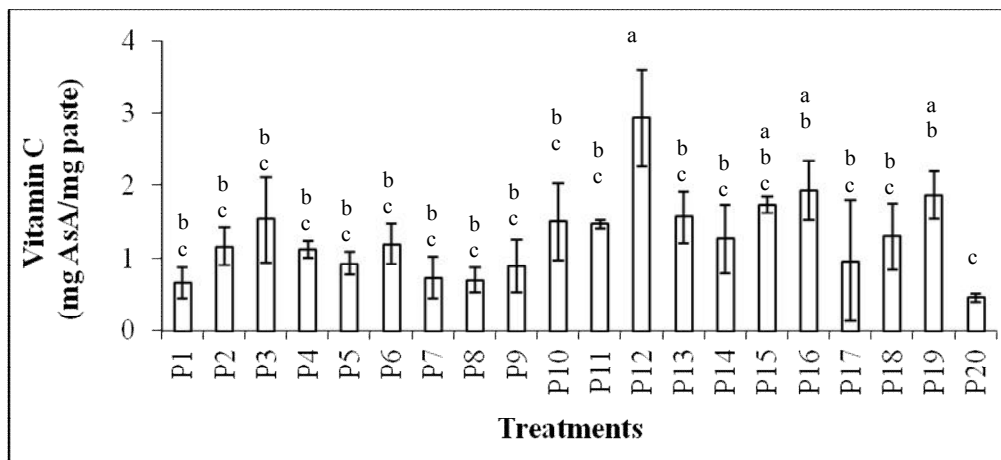


Fig. 7. Levels of Vitamin C in tomato paste as affected by different treatments
P1, P2, P3 ... P20: Labels for Paste different treatment conditions

4. DISCUSSION

Before applying any treatment, the tomato juice and paste had in average pH value readings of 4.6 and 4.7 respectively. These products can be considered as being low-acid foods, and so need sufficient thermal treatments. However, when processing tomatoes, the pH should be lower than 4.4 to avoid potential spoilage with thermophilic organisms [31]. Our results indicated that these pH are not suitable for preservation because they are higher than the pH 4.5 that is the limit to prevent easy growth of most pathogens and toxin production. This little discrepancy with the recommended levels was compensated with the use of essential oil during thermal treatments.

The percentage of microbial reduction was assessed before the beginning of storage at ambient temperature. According to the equations (Equations 1 and 2), no direct effect of the independent variables were observed. In both tomato juice and paste, the percentage reduction of spores depended on the combination of Rosemary essential oil (REO) with spore concentration and heating time with spore concentration. However, these equations (Equations 1 and 2) only explained a maximum of 35% of the variability of the data, indicating that other important factors explaining these reduction are still to be identified. *B. cereus* spores are heat and radiation resistant [32,33]; the heat resistance might vary with different strains [11], pH and water activities of the heating and recovery medium [34], sporulation conditions and the nature of suspending media [32]. Despite the fact that inactivation of bacterial spores requires high temperature and long heating time, food containing $> 10^4$ *B. cereus*/g is not safe for the consumption, as the real infectious dose vary from about 10^5 – 10^8 viable cell or spores/g [11]. Our results have indicated that all treatments in both tomato juice and paste inoculated with 4 log CFU/g have registered a certain level of *B. cereus* reduction, except where there was no heating for deactivation (Tables 3-4). This reduction is a combined effect of heat and rosemary essential oil (REO). Previous studies have demonstrated that the efficacy of REO in inhibiting a variety of pathogens depends on many factors including the plant location and seasonal variations, the phenotype stage of the plant, the method of extraction of the essential oil, the procedure used in the antimicrobial assays, the type of organism, the cultivation conditions (incubation time, temperature,

oxygen), the culture medium, the concentration of the test substance and the solvents used to dilute the oil, among other factors [35,36,37]. Rosemary extracts at a level of 0.06 -1% inhibit the growth of Gram-positive pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* [20].

Regarding total polyphenols, results (Fig. 3) indicate an increase in the total polyphenols content of tomato paste compared to tomato juice (Fig. 2), independently of the treatments (*B. Cereus* spores, REO, cooking time). Thermal processing has been shown to release more bound phenolics due to the breakdown of cellular constituents [24,33,38]. This can explain better the increase in total polyphenols content observed in tomato paste after being subjected to processing techniques.

The loss of vitamin C in different tomato products increase with heating time and number of processing steps [39]. During processing, vitamin C is destroyed mainly due to oxidation reactions and the heat applied in the presence of air; and in addition to the effect of oxygen, such high temperature applications themselves cause oxidative stress. Results in Figs. 6 and 7 are consistent with data from the literature, where numerous examples can be found of vitamin C degradation during thermal processing of tomato products [40,41,42,43]. Loss of vitamin C occurs primarily by chemical degradation that involves oxidation of ascorbic acid to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to form other nutritionally inactive products [24].

Tomato juice typically has a commercial shelf life of 12 months [30] and concentrated tomato paste is typically stored for 1 year or more [44]. The active components of spices at low concentrations may interact synergistically with other factors to increase preservative effect. The findings of Eissa, et al. [45] have demonstrated that the use of volatile oil extracts (lemon grass, clove and rosemary) has been an effective method of quality improvement and shelf life extension in apple juice, stored at 4°C.

5. CONCLUSION

Using *Rosmarinus officinalis* essential oil can help reduce the thermal impact during tomato juice and paste processing even in condition of high contamination of spores of *Bacillus cereus*. Moreover in this processing condition, nutritional

compounds are not significantly impacted. 12 months shelf life can be achieved after high *Bacillus cereus* spores contamination lower or equal 10^4 *Bacillus cereus* spores/g of the raw material by applying Only 10 min appertisation at 95°C in the presence of 100 ppm of ROE in tomato juice while for tomato paste the same concentration of ROE needs 20 min appertisation at 95°C.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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