Article

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Effect of xantham gum, steviosides, clove, and cinnamon essential oils on the sensory and microbiological quality of a low sugar tomato jam

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Abstract

The partial or total decrease of sugar content in the formulation of jams affects their physical, chemical and microbiological stability. In order to minimize these technological problems, we studied the effect of xanthan gum (XG), steviosides, cinnamon (CO), and clove (CLO) essential oils on the sensory and microbiological quality of a low sugar tomato jam. Levels of 0.250 g/100 g steviosides and 0.450 g/100 g XG showed maximum score of overall acceptability of jam. The combination of essential oils produced synergistic and additive effects *in vitro* on growth of *Z. bailii* and *Z. rouxii*, respectively. However, in the jam, CO was more effective and CLO did not modify the CO action. Cell surface was one of the sites of action of CO since a decrease in yeast cell surface hydrophobicity was observed. From the microbiological and sensory points of view, 0.0060 g/100 g CO showed the maximum score of jam overall acceptability and did not cause yeast inactivation but it could be useful as an additional stress factor against yeast post – process contamination. The adequate levels of XG, steviosides, and CO can improve the quality of a low sugar jam formulation.

Keywords

Steviosides, xanthan gum, cinnamon oil, low sugar jam, spoilage yeasts

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INTRODUCTION

Over the past decades, obesity has reached epidemic rates worldwide. Obese people are at increased risk of diabetes, cardiovascular disease, and hypertension, among others chronic diseases (World Health Organization, 2014). These facts led to the development of foods with low sugar content, such as jams. The partial or total decrease of sugar content in jams affects their physical, chemical, and microbiological stability. The decrease in sweetness, body, and mouthfeel, as well as changes in the appearance and the increase in water activity (a_w) of low sugar jams are the technological problems to be solved (Basu and Shivrave, 2010). Reducing the sweet taste may be offset by the addition of sweeteners, while the presence of thickeners may improve mouthfeel and appearance. Moreover, the increase in a_w may affect the microbiological stability of foods, making the addition of preservatives necessary.

The consumer demand for a reduction of synthetic additives' intake promotes the search for natural additives that replace all or part of them in food

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formulations. In this sense, stevia is gaining interest. This sweetener is extracted from the leaves of *Stevia rebaudiana* (Bertoni), an herb native to Paraguay. The extract is 300 times sweeter than sucrose and also has a hypoglycemic response making it attractive for diabetic people (Chatsudthipong and Muanprasat, 2009). Its use as a food additive has been approved in a number of countries, including Argentina, USA, Brazil, Paraguay, China and the European Union. Since then, it is one of the most used sweeteners to replace sugar in food formulations over the world (Prakash et al., 2008; Scott-Thomas, 2013).

The reduction of sugar level necessitates the addition of thickeners that improve mouthfeel and appearance. For this purpose, xanthan gum (XG) is frecuently used. It is a heteropolysaccharide produced by *Xanthomonas campestris*. It is non-toxic and has been approved by the Food and Drug Administration (FDA) for use as a food additive without concentration limits. It is widely used in the formulation of sauces, syrups, salad dressings and jams (García-Ochoa et al., 2000; Sikora et al., 2008).

Some of the hurdles applied to preserve low sugar foods are: the decrease in pH and a_w, thermal treatment and the use of preservatives. These hurdles prevent the growth of pathogens but are overcome by spoilage yeasts, such as *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* (Stiles et al., 2002). These yeasts are osmophilic, acid tolerant, and preservative resistant (Gliemmo et al., 2006). They are able to grow under anaerobic atmosphere, exhibit minimum nutritional needs, and are resistant to pasteurization and to cleaning agents. As a consequence, their growth leads to significant economic losses (Deak, 2008; Loureiro and Querol, 1999; Praphailong and Fleet, 1997). Therefore, the control of yeast growth in acidic products is of interest.

The use of essential oils as natural preservatives in foods has reemerged. Some essential oils have been used in medical treatments.

The antimicrobial action of essential oils is attributed to the penetration of lipophilic components through the membrane to the site of action within the cell (Lv et al., 2011). Cinnamon (CO) and clove oils (CLO) inhibit the growth of molds, yeasts and bacteria *in vitro* and *in vivo* (Conner and Beuchat, 1984; Goñi et al., 2009; Matan et al., 2006; Prabuseenivasan et al., 2006). However, the information about the use of these oils against yeasts growth is scarcely reported.

The replacement of sucrose by thickeners, sweeteners and preservatives in low sugar jams needs the sensory quality assessment to minimize the differences with regular jams and to avoid an unacceptable sensory impact. The addition of low methoxyl pectin and the mixture aspartame/acesulfame-K to a low calorie fruit jelly did not affect the overall acceptability (Acosta et al., 2008). Carrageenan addition improved the mouthfeel of a low-calorie Christophene jam (Gajar and Badrie, 2001).

The presence of essential oils may produce undesirable flavors in foods. Few studies measure the inhibitory concentrations of essential oils and analyze their effect on the sensory properties of food. A level of 0.25 µl/ml lemon essential oil extended the shelf life of clear apple juice and exerted a positive influence on its taste (Tserennadmid et al., 2011). Carrot slices treated with oregano, marjoram, basil and lemon balm solutions were sensory acceptable but only oregano and marjoram treatments had positive scores of overall acceptability for lettuce (Gutierrez et al., 2008). To reduce the sensory impact of the essential oil, the use of 1% v/w CLO in combination with low temperature has been proposed to preserve chicken frankfurters (Mytle et al., 2006).

In order to minimize the technological problems arising from the formulation of low sugar jams, we studied the effect of natural additives on sensory properties and microbiological stability of a low sugar tomato jam. For that purpose, on a first stage, the effect of XG and steviosides was evaluated on overall acceptability of the jam by the use of a full factorial design. On a second stage, antimicrobial activity of CO and CLO was assayed against spoilage yeasts *in vitro* and in the jam containing the optimal sensory levels of XG and steviosides. Finally, the sensory and microbiological quality of the jam containing different levels of CO was studied.

MATERIALS AND METHODS

Materials

Glucose, citric acid and calcium chloride were from Merck Química (Argentina, Argentina); vanilla, CO (*Ceylon Type, natural identical*), CLO (*Syzygium aromaticum*), and 2,3,5-triphenyltetrazolium chloride were from Sigma-Aldrich (USA), and glycerol was from Sintorgan S.A. (Argentina). All of them were of reagent grade. Steviosides (90 g/100 g of a mixture of steviosides and 10 g/100 g maltodextrin) was from Inmobal Nutrer (Argentina), xylitol was from Gelfix (Argentina), and xanthan gum and low methoxyl pectin were from Cargill (Argentina). All of them were of food grade. All culture media used for microbiological evaluations (SB, Sabouraud broth; SA, Sabouraud agar; bacteriological agar; PCA, plate count agar; MRS, de Man, Rogosa and Sharpe agar; and VRBL, violet red bile lactose agar) were from Biokar (Biokar Diagnostics, Beauvais, France).

Processing of jam

Fresh tomatoes (*Lycopersicum esculentum*), purchased from the local market (Buenos Aires, Argentina), were

washed, peeled off, de-seeded, and cut into cubes. Five jams were prepared according to a 2^2 full factorial design with two variables (XG and steviosides) at two levels and a central point (Table 1) in three blocks. The remaining composition is shown in Table 2. The jams were elaborated following the general processing steps showed in Figure 1. The sugar content was reduced by the addition of xylitol and glucose, reaching 30 ° Brix at the end of cooking. This value includes 10 g/100 g assimilable carbohydrates and 20 g/100 g xylitol, which have caloric values of 4.0 and 2.4 kcal/g, respectively.

The total soluble solids content were recorded during the process with a refractometer (Westover, China). The pH was adjusted to 3.50 by addition of citric acid. It was determined with a pH meter Solution Analyzer 5800-05 (Cole-Parmer, Chicago, IL, USA) provided with a glass electrode. The pasteurization was performed in an electric sterilizer (Tuttnauer 3150, Israel).

Effect of xanthan gum and steviosides on overall acceptability of a tomato jam

A group of 100 consumers (35 males and 65 females) of low sugar jams evaluated the overall acceptability of jams at three days after elaboration using a balanced verbal 9-point hedonic scales (1 = dislike very much; 9 = like very much) (Meilgaard et al., 1987). Assessors received the samples at room temperature, served in coded containers (10 g), in randomized order and in three replicated sessions as it was recommended by Meilgaard et al. (1987).

Yeasts strains and inoculum preparation

Zygosaccharomyces bailii NRRL 7256 and *Zygosaccharomyces rouxii* ATCC 28.253 inocula were prepared separately. The strains were stored at -20.0 ± 0.5 ° C in SB plus 10.0 g/100 g glycerol. Before their use, they were grown twice in SB at 25.0 ± 0.5 ° C during 24 h. After that, each inoculum was diluted in peptone water (1.5 g/100 g) to reach 0.5 McFarland units, corresponding to a population of approximately 10^6 CFU/ml.

Screening for antimicrobial activity of essential oils

The agar disk diffusion method was used (Lv et al., 2011). Plates containing solidified SA were seeded on a surface with 1 ml of the diluted inocula of yeasts. Then, sterile blank filter disks (6 mm diameter, Oxoid) were applied on the surface and seeded with 10 µl of each essential oil. After 24 h of incubation at

Table 1. Levels (g/100 g) of xanthan gum (XG) and steviosides in jams in the factorial design.

Concentration XG	Steviosides
0.450	0.000
0.225	0.125
0.000	0.000
0.000	0.250
0.450	0.250

Table 2. Jam composition (g/100 g).

Ingredient[a]	Concentration
Xylitol	20.00
Glucose	3.00
Low methoxyl pectin	0.60
Vanilla	0.05
$CaCl_2$	0.025

[a]Different levels of xanthan gum and steviosides were also added depended on the formulation evaluated.

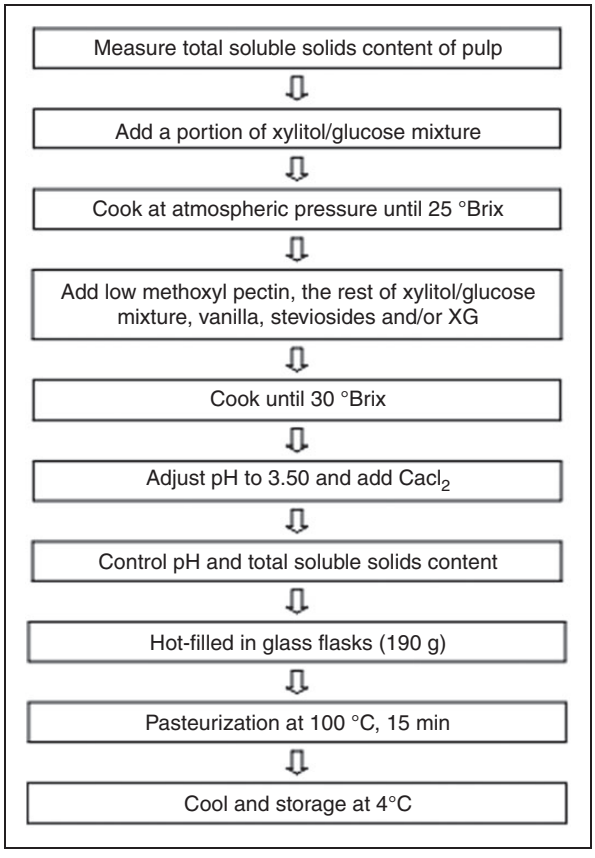

Figure 1. General processing steps in the jam production.

25.0 ± 0.5 ° C, the diameter of inhibition zones including the disks was measured with a caliper.

Determination of minimum inhibitory concentrations of essential oils

The tube dilution method was assayed in triplicate (O'Bryan et al., 2008). CO and CLO solutions, prepared in SB containing 0.15 g/100 g bacteriological agar, with the pH adjusted to 3.50 by citric acid addition, were tested in the range from 0.0014 to 0.1812 g/100 g. Negative and positive controls were tested in parallel, being the former no inoculated SB, and the latter inoculated SB free of antimicrobials. Each tube, containing 3.5 ml of system, was inoculated with 50 µL of the diluted inoculums. The tubes were incubated at 25.0 ± 0.5 ° C for five days. Then, the minimum inhibitory concentrations (MICs) were obtained as the lowest concentration at which no turn color of the indicator 2,3,5-triphenyltetrazolium chloride was observed after incubation, i.e. microbial growth inhibition compared with positive and negative controls (National Committee for Clinical Laboratory Standards, 1999).

Evaluation of essential oils interactions

The macro dilution checkerboard method was used in triplicate and the fractional inhibitory concentration indices (FIC) were calculated. The technique proposed by Tserennadmid et al. (2011) was used with some modifications. CO and CLO solutions, the range of concentrations studied (8 at 1/8 of the MIC values), and the controls were the same as those used in the determination of MICs. Serial two-fold dilutions of each antimicrobial solution were mixed in a matrix of 10 ml sterile tubes so that each row or column contained a fixed amount of the first antimicrobial and increasing amounts of the second one. Each matrix also contained a row and a column in which each antimicrobial was present alone (Singh et al., 2000). Each tube, containing 3.5 ml of system, was inoculated with 50 µL of the diluted inoculum. The tubes were incubated at 25.0 ± 0.5 ° C for five days and then evaluated for their microbial growth.

The MIC of each antimicrobial, alone (MIC_A or MIC_B) and in combination ($MIC_{A\text{-}B}$ or $MIC_{B\text{-}A}$), was used to calculate the FIC index as follow

$$FIC = \frac{MIC_{A-B}}{MIC_A} + \frac{MIC_{B-A}}{MIC_B}. \quad (1)$$

The type of interaction between the antimicrobials can be determined considering the FIC index value. An FIC index value near to 1 indicates addition; if it is less than 1, synergy; and if it is greater than 1, antagonism (Lopez Malo Vigil et al., 2005).

Evaluation of essential oils activity in the tomato jam

A tomato jam was elaborated (Figure 1) with the composition given in Table 2 and the addition of 0.250 g/100 g steviosides and 0.450 g/100 g XG. The jam was fractionated in glass flasks (190 g) to which were added, in triplicate, the MICs values of CO (0.0112 and 0.0028 g/100 g for *Z. bailii* and *Z. rouxii*, respectively), the MIC of CLO (0.0453 g/100 g for both yeasts), 0.0400 g/100 g CO and the mixture 0.0400 g/100 g CO with the MIC of CLO. A control system free of oils was prepared for comparison purposes. After pasteurization and cooling, jams were inoculated with *Z. bailii* and *Z. rouxii* separately to a level of 10^3 CFU/g. The flasks were stored at 15.0 ± 0.5 ° C simulating a post-process contamination and storage under inadequate refrigeration. Throughout the storage, jam samples (5 g) were aseptically taken from each flask and placed in sterile bags containing 45 ml of 0.1 g/100 g peptone water. Then, the viable population of yeasts was determined by surface plate count on SA after five days of incubation at 25.0 ± 0.5 ° C.

Effect of CO on overall acceptability of the tomato jam

The tomato jam containing 0.250 g/100 g steviosides and 0.450 g/100 g XG was fractionated and CO was added obtaining the following levels: 0.0000 – 0.0060 – 0.0110 – 0.0130 – 0.0400 g/100 g. After pasteurization and cooling, the jams were stored at 5.0 ± 0.5 ° C until use.

The overall acceptability of the low sugar jams was evaluated by a group of 100 consumers (35 males and 65 females) of low sugar jams. The procedure and conditions were similar to the used in the previous test. This assay allowed the selection of two sensory acceptable oil levels, which were evaluated microbiologically as described in the next section.

Effect of CO on microbiological stability of the tomato jam

The simulation of a post-process contamination with the spoilage yeasts was assayed. Jam samples free of CO and containing 0.0060 and 0.0110 g/100 g CO were inoculated with *Z. bailii* and *Z. rouxii* separately reaching a level of 10^3 CFU/g. The samples were stored at 5.0 ± 0.5 ° C and the viable population of yeasts was determined at 0, 7 and 20 days of storage.

Moreover, the level of indigenous flora was determined to control the microbiological stability.

The jams were exposed uncovered for 30 min at laboratory bench, stirred, closed and stored at $5.0 \pm 0.5^\circ$ C. Then, aerobic mesophilic, lactic acid, and coliform bacteria were investigated in PCA, MRS agar, and VRBL agar, respectively. The plates were incubated at $30.0 \pm 0.5^\circ$ C for 48 h. The yeasts and molds were determined by surface plate count on SA after five days of incubation at $25.0 \pm 0.5^\circ$ C.

CO chemical analysis

Quantitative and qualitative analysis of the most effective oil (CO) were performed by GC-FID-MS using a Perkin Elmer Clarus 500 GC-FID-MS system with a special configuration, equipped with a single split-splitless injector connected by a flow splitter to two capillary columns: a polyethylene glycol MW *ca*. 20.000 column and a 5% phenyl-95%-methyl silicone column, both $60\,m \times 0.25\,mm$ with 25 µm of fixed phase (J&W Scientific). The methodology proposed by Gil et al. (2007) was used.

Cell surface hydrophobicity

In order to check the possible effect of CO on cell surface, cell surface hydrophobicity was determined using the Microbial Adhesion to Hydrocarbon Test (Li and McLandsborough, 1999; Rosenberg and Gutnick, 1980). The methodology proposed by Gliemmo et al. (2013) was used. Briefly, aliquots of systems containing one-half of the MIC values of CO were inoculated with *Z. bailii* and *Z. rouxii*, separately and they were incubated at $25.0 \pm 0.5^\circ$ C for 24 h in order to obtain log phase cells. After that, the systems were centrifugated (10,000 rpm for 10 min). Pellets were washed twice and resuspended in Ringer's solution at pH 3.50, reaching a turbidity greater than the 0.5 Mc Farland standard. Four milliliter aliquots were dispensed into two tubes. One milliliter of xylene was added to one of them (*Am*), while the other was used as control (*Ac*). After 10 min of incubation, tubes were vortexed for 1 min, and kept at room temperature for 30 min for phase separation. Then, 2.00 ml of the aqueous phase was removed and the optical density (600 nm) was determined using a spectrophotometer (Shimadzu UV-1203, Japan), which was zeroed using Ringer's solution at pH 3.50. The determinations were made in triplicate. The absorbance of the microbial assay tubes (*Am*) and the absorbance of the control (*Ac*) were used to calculate the percentage of cell surface hydrophobicity of yeasts as

$$\% = \frac{Ac - Am}{Ac} 100. \qquad (2)$$

Data analysis

Experimental data obtained from the full factorial design were subjected to a multiple regression analysis to fit the following first-order regression model

$$Y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_1 x_2 + \varepsilon \qquad (3)$$

where Y is the average score of overall acceptability; $\alpha_{0,1,2,3}$ are the regression coefficients for the intercept, linear, and interaction coefficients, respectively; $x_{1,2}$ are the independent variables (x_1, level of steviosides and x_2, level of XG), and ε is the error term.

The adequacy of the regression model generated by the factorial design was examined by analysis of variance (ANOVA) at 5% significance level, adjusted correlation coefficients (R^2), and the absolute average deviation (AAD) (Baş and Boyaci, 2007). Also, ANOVA and *p*-value were used to evaluate the significance of the linear and interaction terms of each model.

Analysis of variance and the Least Significant Difference (LSD) test were applied to establish differences between diameters of inhibition halos, overall acceptability scores, and cell surface hydrophobicity values.

In all cases, statistical significance was evaluated at a 5% level ($\alpha = 0.05$) and the analyses were performed using Statgraphics Plus for Windows, version 5.1 (Manugistics, Inc., Rockville, MD, USA).

RESULTS AND DISCUSSION

The water activity of jams was 0.952 ± 0.002, the pH value was 3.50 ± 0.02, and the total soluble solid content was $29.5 \pm 0.5^\circ$Brix. These values were kept constant along 35 days of storage under refrigeration conditions.

The energetic value of jams was 17.6 kcal per 20 g serving. This value represents the 33% of the caloric value of regular jams.

Effect of xanthan gum and steviosides on overall acceptability of a tomato jam

The first-order regression model for average scores of overall acceptability was fitted using the experimental data. The regression equation was

$$\begin{aligned} Overall acceptability = {} & 4.48 + 1.26(XG) \\ & + 1.33(Steviosides) \\ & + 7.70(XG)(Steviosides) \end{aligned} \qquad (4)$$

where (*XG*) and (*steviosides*) are the levels of these additives expressed in g/100 g.

The adj R^2 was 0.94, AAD was 3.63, and p-values were lower than 0.002 for XG, steviosides, and the interaction coefficients. The adj R^2 and the AAD values obtained indicate a good correlation between the observed and the predicted values of responses and that the model gives a reasonably good estimation of responses in the studied range.

In the absence of steviosides, the presence of XG increased overall acceptability of jams (Figure 2). The addition of steviosides to a system free of XG increased the overall acceptability (Figure 2).

The joint presence of XG and steviosides significantly increased overall acceptability ($p = 0.002$). This trend is shown in Figure 2 where the addition of XG to the jam containing 0.250 g/100 g steviosides increased the overall acceptability in one unit.

It was reported that the increase in pectin concentration decreased the taste of a mango jam (Basu and Shivrave, 2010). The acceptability of a low calorie jelly of fruits increased by incrementing the level of a mixture of sweeteners (aspartame/acesulfame K/sorbitol) or the concentration of low methoxyl pectin (Acosta et al., 2008).

Levels of XG and steviosides showing appropriate values of overall acceptability will allow to compensate the decrease in sweetness, body, and mouthfeel produced by the reduction of sugar content in jams. Thus, the jam containing 0.250 g/100 g steviosides and 0.450 g/100 g XG showed the highest score of overall acceptability (6.2).

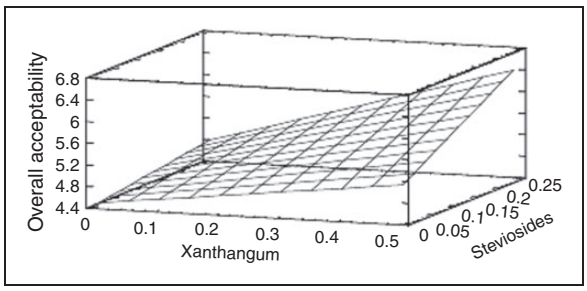

Figure 2. Estimated response surface graph for the combined effect of steviosides and xanthan gum (XG) levels on overall acceptability of jams.

Table 3. Diameters of growth inhibition halos (mm) and minimum inhibitory concentrations (MIC) (g/100 g) of cinnamon (CO) and clove oils (CLO).

	Diameter of halo[a]		MIC	
Essential oil	*Z. bailii*	*Z. rouxii*	*Z. bailii*	*Z. rouxii*
CO	53.0 ± 0.5^a	55.0 ± 0.7^a	0.0112	0.0028
CLO	23.5 ± 0.7^b	20.0 ± 0.8^b	0.0453	0.0453

[a]Errors represent standard deviation.

Values followed by the same letter are not significantly different ($\alpha = 0.05$).

In vitro antimicrobial activity of essential oils

Both antimicrobials showed inhibitory action on the growth of yeasts being the CO more effective than the CLO (Table 3). The MIC values of essential oils depended on the antimicrobials that were tested and the yeast target (Table 3). CO was the most effective in inhibiting growth, since it showed the smallest MIC values. Furthermore, both yeasts showed equal sensitivity to CLO, but *Z. bailii* was more resistant to CO than *Z. rouxii*. Probably, *Z. bailii* would be more resistant than *Z. rouxii* to preservatives' action as it was also reported at acidic pH values for lipophilic preservatives (Praphailong and Fleet, 1997).

CO and CLO are effective in inhibiting the growth of yeasts and bacteria. Levels of CO and CLO ranging from 0.0100 to 0.0200 g/100 g diminished the growth of some food spoilage yeasts (Conner and Beuchat, 1984). In general, the inhibitory action of CO against bacteria is greater than CLO but there is no information about yeasts (Goñi et al., 2009; Prabuseenivasan et al., 2006). The sensitivity to the action of essential oils may be different among genera of yeasts and between yeasts and bacteria (Conner and Beuchat, 1984).

The combined use of essential oils showed interactions that depended on yeast target and level of antimicrobials. A synergistic effect was observed on *Z. bailii* growth ($FIC_I = 0.75$) by the joint presence of 0.0028 g/100 g CO together with 0.0227 g/100 g CLO and 0.0056 g/100 g CO together with 0.0113 g/100 g CLO. Regarding the action on *Z. rouxii*, an additive effect was observed ($FIC_I = 1.00$). No information is available about the effect of combined use of CO and CLO on yeast growth in aqueous media. However, few studies in vapor phase indicate that the interactions depend on essential oil concentration and the target microorganism (Goñi et al., 2009; Matan et al., 2006).

Antimicrobial activity of essential oils in a tomato jam

Initially, the growth of yeasts was not modified by the presence of oils. The concentration equal to the MICs in broth slightly decreased yeast populations during storage (Figure 3). As it was observed, CO was more effective than CLO in the inhibition of yeasts' growth in broth, showing the lowest inhibitory levels. Then, it was decided to assay a higher level of CO (0.0400 g/100 g) in the jam and its combination with the MIC of CLO.

The presence of 0.0400 g/100 g CO concentration significantly diminished the growth of both yeasts.

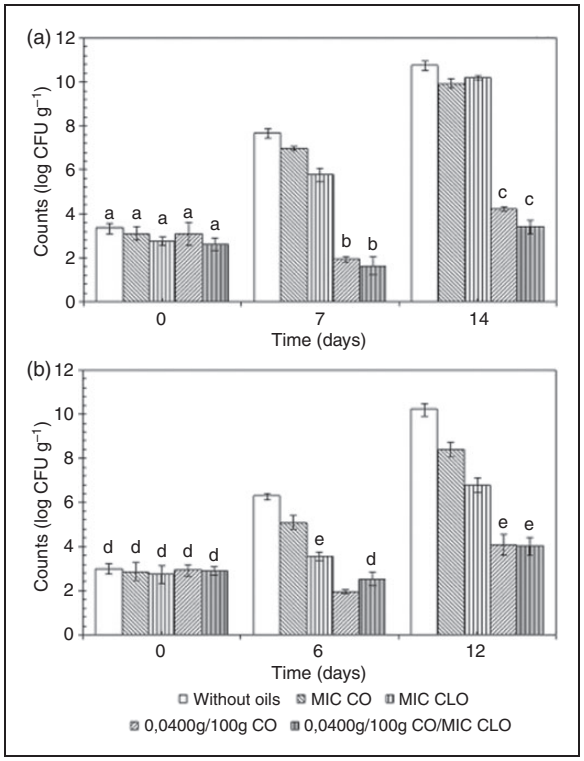

Figure 3. Effect of cinnamon oil, clove oil, and their mixture on yeast populations in the tomato jam stored at 15 °C. *Z. rouxii* (a), *Z. bailii* (b). Columns with the same letter are not significantly different (α= 0.05). Columns without letters are significantly different (α= 0.05). MIC: minimum inhibitory concentration.

The population of *Z. rouxii* decreased in 6 log cycles after 7 and 14 days of storage (Figure 3a) whereas *Z. bailii* decreased in 4 and 6 log cycles after 6 and 12 days of storage, respectively (Figure 3b).

Reductions in yeasts population observed in the jam containing 0.0400 g/100 g CO and the MIC of CLO were similar to those observed in the presence of 0.0400 g/100 g CO, indicating the absence of interaction between the oils. These results highlight the environmental influence on the oils antimicrobial activity. On one hand, the levels of essential oils with inhibitory action in laboratory media did not achieve the same effect in the jam. This is in agreement with previous studies (Lv et al., 2011; Tserennadmid et al., 2011). On the other hand, the interaction effects observed in broth were not observed in the jam. The structure of food matrix may act as an additional factor that influences either the growth of the microorganisms or the functionality of the preservatives. Cloudy apple juice protected *Saccharomyces cerevisiae* against the effect of lemon essential oil, while in clear apple juice it had an inhibitory action (Tserennadmid et al., 2011). Yeast cells may adhere to juice particles reducing the action of lemon oil. The nisin effectiveness against *Lactobacillus fructivorans* was disturbed by the presence of Tween 20 and oil in model salad dressings (Castro et al., 2009).

Since no interaction was observed between CLO and CO on yeasts inhibition in jam and being CLO less effective than CO, the following assays were conducted with the latter.

Effect of CO on overall acceptability of the tomato jam

Overall acceptability of jams containing the CO level that exerted antimicrobial activity (0.0400 g/100 g) and levels of CO close to MIC values were evaluated. The lower concentrations assayed were selected because 0.0400 g/100 g may produce undesirable impact on the acceptability of the jam. After that, a few levels of CO were selected and the microbial stability of the jams was assayed.

The overall acceptability of jams containing different levels of CO is shown in Table 4. The scores were significantly different ($p = 0.0154$). Considering that the midpoint of the scale ("I do not like nor dislike") corresponds to a score of 4.5, those jams with overall acceptability values above 5.0 were considered as "acceptable". Therefore, all jams were acceptable with the exception of the jams containing 0.0400 and 0.0130 g/100 g CO. No significant differences were observed among the jams free of CO and the one containing 0.0110 g/100 g oil. The formulation containing 0.0060 g/100 g CO had the highest score and it was significantly different from the rest of the jams.

Sensory acceptable formulations (0.0000 – 0.0060 – 0.0110 g/100 g CO) were submitted to microbiological evaluation.

Effect of CO on microbiological stability of the tomato jam

In the absence of CO, counts of *Z. rouxii* and *Z. bailii* remained constant throughout the storage (Table 4). The addition of 0.0060 g/100 g CO decreased yeasts' growth at 20 storage days. In jams containing 0.0110 g/100 g CO, the growth of *Z. rouxii* diminished at 20 days while *Z. bailii* growth decreased at seven storage days (Table 4).

The counts of indigenous flora were lower than 9 CFU/g in the selected formulations and they remained at that level after 35 days of storage.

The hurdles applied in the formulation of the jams were the decrease in pH through citric acid addition, a_w depression by the presence of glucose and xylitol, CO addition, the thermal treatment, and the storage at 5 °C. These factors were suitable as indigenous flora did not grow during storage, indicating that the jams were elaborated in safe conditions. It is noteworthy that,

Table 4. Overall acceptability and yeasts growth (CFU/g) in tomato jam containing different levels of cinnamon oil (g/100 g).

		Yeast growth at different storage time (days)					
		Z. rouxii			*Z. bailii*		
CO level	Overall acceptability	0	7	20	0	7	20
0.0000	5.0[a]	8.7×10^2	7.2×10^2	6.3×10^2	9.8×10^2	6.0×10^2	6.9×10^2
0.0060	6.2	9.0×10^2	6.1×10^2	$<9.0 \times 10^1$	8.4×10^2	3.1×10^2	9.0×10^1
0.0110	4.9[a]	9.0×10^2	6.3×10^2	$<9.0 \times 10^1$	8.8×10^2	9.0×10^1	$<9.0 \times 10^1$
0.0130	4.5	–	–	–	–	–	–
0.0400	1.3	–	–	–	–	–	–

Note: Means in columns followed by the same superscripts are not significantly different ($p < 0.05$).

although in the absence of CO the others factors ensured the microbiological stability of the jam, CO addition may be useful in a situation of contamination with resistant yeasts to the stress factors applied. That is the case of *Z. bailii* and *Z. rouxii* (Loureiro and Querol, 1999). The essential oil levels were not high enough to cause death of microorganisms, but they were sufficient to produce the stasis of growth.

The use of steviosides, XG and CO in a low sugar tomato jam may provide a safe and sensory acceptable formulation contributing to the development of safe and healthy food.

CO effectiveness related to the chemical composition and the cell surface hydrophobicity

The chemical composition of CO is shown in Table 5. Fifteen compounds were identified, representing 96.8% of the total oil. The major constituent was (*E*)-Cinnamaldehyde (78.7%) followed by Eugenol (5.4%).

The effectiveness of CO is particularly attributed to the inhibitory action of cinnamaldehyde. Its action against bacteria, yeasts, and molds has been reported. It has been suggested that the carbonyl group of cinnamaldehyde would bind to proteins affecting the action of amino acid decarboxylases in *Enterobacter aerogenes* (Ali et al., 2005; Burt, 2004; Matan et al., 2006). Also, the minor components may interact with major components resulting in additive or synergistic effects. The mixture of cinnamaldehyde and eugenol synergistically acted inhibiting the growth of some pathogenic bacteria (Burt, 2004).

On the other hand, CO addition decreased the percentage of cell surface hydrophobicity of yeasts from 91 ± 3 to 57 ± 1 for *Z. rouxii* and from 86.0 ± 0.1 to 44 ± 4 for *Z. bailii*. It was proposed that a change in the amount of cells that can adhere to a solvent is an expression of a change in surface structure (Ming and Daeschel, 1995).

Table 5. Chemical composition of cinnamon essential oil.

Compounds	g/100 g	RI no polar
α-Pinene	0.9	945
β-Pinene	0.6	994
α-Phellandrene	0.6	1017
ρ-Cymene	1.6	1031
Limonene	2.4	1034
1,8-Cineole	2.0	1041
Linalool	0.8	1107
Camphor	0.2	1172
α-Terpineol	0.7	1211
γ-Terpineol	0.3	1215
(*Z*)-Cinnamaldehyde	0.2	1244
Linalool acetate	0.6	1252
(*E*)-Cinnamaldehyde	78.7	1303
Eugenol	5.4	1360
β-Caryophyllene	1.8	1437
TOTAL	96.8	

Note: RI, retention indices relative to C_6-C_{24} n-alkanes on the column.

The mode of antimicrobial action of essential oils is related to their interaction with the lipids of cell membrane and the mitochondria increasing its permeability, causing swelling and reducing membrane function. Also, lipophilic compounds of essential oils may interact with hydrophobic sites of proteins located in the cytoplasmic membrane (Burt, 2004; Sikkema et al., 1994). Furthermore, the effectiveness of these interactions depends on the range of hydrophobicity of essential oils since aqueous solubility is a limiting factor of the accumulation of hydrophobic compounds in the cell membrane to lethal levels (Goñi et al., 2009).

No information about the relation between the effect of CO or cinnamaldehyde on yeast cell membrane and changes in the hydrophobicity of cells has been found.

In the present study, the interaction between CO and yeasts cell membrane may be the cause of the drop in the percentage of cell surface hydrophobicity of yeasts.

CONCLUSIONS

Levels of XG and steviosides showing appropriate values of jam overall acceptability were found. This would allow compensating the decrease in sweetness, body, and mouthfeel produced by the reduction of sugar content in the jam. The essential oils assayed showed synergistic and additive effects *in vitro* on growth of *Z. bailii* and *Z. rouxii*, respectively. However, in the jam, CO was more effective and CLO did not modify the CO action. Cell surface was one of the sites of action of CO. It was found a suitable level of CO from the microbiological and sensory points of view. Although this level did not cause yeast inactivation, it could be useful as an additional stress factor against yeast post-process contamination. The adequate levels of XG, steviosides, and CO found may improve the quality of a low sugar jam formulation.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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