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Evaluation of Chitosan Acid Salts as Clarifying Agents of Orange Nectar

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Abstract

The use of chitosan acid salts was assessed in the clarification orange nectar. Clarification process was performed at laboratory scale by the classical methodology of jar test by adding chitosan acetate or chitosan lactate at concentrations between 0 g/L and 2 g/L of nectar. Numerical optimization method was used through an IV Optimal response surface for the best variant of coagulation-flocculation based on clarification yield. No significant effect of the salt type and concentration or clarification time on the physicochemical parameters of nectars were observed. Both chitosan acid salts could be used as clarifying agents, although chitosan lactate at 1 g/L was more effective, obtaining the highest yield (66.14%) to a time of 90 min. In general, there was no significant influence of the type and concentration of chitosan acid salt on the sensory attributes of the products and nectar clarified with chitosan lactate showed the higher overall quality.

Keywords: Chitosan acetate; Chitosan lactate; Clarification; Orange nectar

Introduction

The increasing in the consumption of juices, nectars and beverages is promoted by the new living styles and eating habits related with the healthy properties of this type of products [1]. The turbidity of juices and nectars are associated with the presence of colloidal suspensions of cellular components with varying amounts of small pieces of tissue, where the solids content is generally between 5% and 20% (w/w) [2]. In many instances, this turbidity is not striking in certain products. So that, some methods as clarification can be achieved to decrease the turbidity.

Clarification by physical [3,4] enzymatic [2] and chemical treatments or a combination of them [5] are commonly methods in the processing of fruit juices and nectars. Clarification through clarifying agents such as gelatin, bentonite, silica sol and polyvinyl pyrrolidone or a combination of these compounds was reported [6]. Another alternative is the use of chitosan, a nontoxic and biodegradable agent [7] that for its polycationic feature, has been successfully employed in the clarification of wine [8], juices of apple [9-12], grape, lemon, orange [9], pear [13] and green tea [14]. The use of chitosan in this respect is hindered due to its solubility in organic acids, that´s why, in the present work it is proposed the use of chitosan acid salts due to their water solubility.

In this sense, and considering the potential of chitosan as clarifying agent in fruit and vegetable juices and nectars, the present work aimed the effect of concentration of chitosan acetate and chitosan lactate as well as the agitation time during the clarification process on the physicochemical and sensory properties of an orange nectar.

Materials and Methods

Preparation of nectars

Orange nectars were prepared from concentrated orange pulp (65º Brix and acidity between 1.9% and 3.5% w/w of citric acid) produced by the Citrus Jagüey Grande Co. (Matanzas, Cuba) belonging to the Cuban Ministry of Food Industry. Concentrated orange pulp (10%) was mixed with other ingredients (water, sugar and citric acid) according to the recommendations indicated by the NC 340 and Horváth-Kerkai [15,16].

Experimental design for clarification process

Concentration of chitosan acid salts and clarification time were selected as factors. The Design Expert 8.0.6 software (Stad-ease Inc., 2011) was used to generate the experimental design matrix (Table 1) and processing the data from each run. Numerical optimization method was used through an IV Optimal response surface for the best variant of coagulation-flocculation based on clarification yield, generating a mathematical model that describes the changes in volume of clarified juice, adjusted as follows:

$$\text{Yield (\%)} = a_0 + a_1A + a_2B + a_3AB + a_5A^2 + a6B^2 \quad \text{[Equation 1]}$$

Where a0-a6, coefficients obtained by the model matrix resolution; A, concentration of chitosan acid salt, chitosan acetate or chitosan lactate; and B, clarification time.

Clarification process

It was used chitosan acetate and chitosan lactate (30% m/m of chitosan), produced at Drug Research and Development Center (Havana, Cuba) from chitosan from chitin of common lobster (*Panulirus argus*) of molecular weight of 275 kDa and degree of deacetylation of 75% [17,18].

Run	Factor	
	A: salt concentration (g/L)	B: clarification time (min)
1	0	90
2	0	60
3	0	120
4	1	90
5	1	90
6	1	90
7	1	60
8	1	90
9	1	120
10	1	90
11	2	60
12	2	120
13	2	90

Table 1: Matrix of experimental design for clarifying process.

The clarification process of orange nectars was performed at laboratory scale by the classical methodology of jars test by using a laboratory Floculator with six units of simultaneous treatment. Each unit has a standard paddle stirrer with a speed controller for fast or slow mixing stages of coagulation and flocculation. It also has a timekeeper and an illuminated screen in the back part for observing the appearance of the samples.

For each experimental run, the appropriate volume of nectar was poured in a glass container of 1 L of capacity and 10 cm of inside diameter. The nectar was stirred at 120 rpm and then the chitosan acid salt was added at 1 and 2 g/L and the mixtures were stirred during different times (60min, 90min and 120 min) at 120 rpm according with the experimental design. After, the agitation was stopped and the mixture was allowed to settle during two hours. Finally, the clarified portion was separated and the clarification yield was calculated and expressed as percentage of clarified nectar respect to the total volume of nectar added in the glass containers before the clarification.

Clarified nectars were packaged in green glass bottles of 330 ml of capacity and storage in refrigeration until their analysis.

Physicochemical evaluation of nectars: Before and after the clarification process, the nectars were evaluated, according with the standards of quality specifications for nectars [16] by determining titratable acidity [19] expressed as percentage w/w of citric acid, soluble solids [20] and pH [21]. All determinations were performed in triplicate.

Sensory evaluation of nectars: The descriptors generation process was performed using seven semi-trained judges such products, by controlled association method [22]. The elimination of terms was made in open discussion with the judges following the criteria reported in the ISO 11035 [23]. Sensory descriptors of the products were evaluated on a structured scale of 10 cm bounded at both ends with increasing intensity of the descriptor from left to right as indicated by the Quantitative Descriptive Analysis [24].

Analysis of results: Analysis of variance was performed using STATISTICS software (version 7, 2004, Stat Soft. Inc., Tulsa, USA) and the Duncan's multiple range test was used for comparing differences among mean values. The significance was defined at $p \leq 0.05$.

Results and Discussion

Evaluation of reference orange nectar

The quality of orange nectar is primarily influenced by microbiological, enzymatic, chemical and physical factors, which are usually those involving the organoleptic characteristics (aroma, flavor, color, consistency, stability and turbidity, separation of the solid/liquid phases) and nutritional characteristics. The physicochemical parameters vary depending on the variety of fruit, its maturity and the climate where it is grown.

Table 2 presents the values of the physicochemical parameters of the formulation of the orange nectar made from a concentrated pulp and used as reference in the present work. These values corresponded to the quality specifications issued for orange nectars [16] except for the acidity. The specifications pointed out that this type of products must contain at least 25% (w/w) of fruit pulp, pH value between 3.5 and 3.9, soluble solids percentage between 14 and 16% and acidity between 0.32 and 0.38% (w/w) expressed as citric acid [16].

Parameter	Mean (Standard deviation)
pH	3.6 (0.5)
Acidity (Percentage w/w citric acid)	0.4 (0.1)
Soluble solids (ºBrix)	16.0 (0.0)

Table 2: Physicochemical parameters of orange nectar of reference (n=3).

Evaluation of clarified nectars

Physicochemical evaluations: Table 3 shows the results of physicochemical parameters of the clarified orange nectar. It is noted that these parameters are within the values set in the [16], being the acidity between 0.26% and 0.45% (w/w) of citric acid, soluble solids between 15.32° and 16.0° Brix and pH between 3.60 and 3.91. Kelebek et al. reported pH values between 3.05 and 3.35 for orange (Citrus sinensis) juice, while Martin-Diana et al. reported values between 3.2 and 4.2 for orange juice fortified with chitin [25,26].

In addition, no significant impact on the type and concentration of salt or clarification time. Chatterjee et al. informed similar results for the clarification of juices of apple, grape, lemon and orange with bentonite, gelatin and chitosan from shrimp, and in none of the cases reported a significant influence of clarifying agent on acidity and soluble solids [9].

On the other hand, Castro et al. evaluated the influence of chitosan concentration, pH, agitation speed and time in the clarification of passion fruit juice, without significant influence on soluble solids [27]. Imeri and Knorr and Einbu and Varum reported the ability of chitosan to reduce the acidity of the fruit juices, because at pH below 6.5, chitosan is positively charged, allowing join acids [28,29]. These results

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were not shown in this study, which could be attributed to the chitosan acid salts with 30% (w/w) of chitosan and their concentration was less than that reported by other authors for chitosan in the use of chitosan in clarification processes [9,27].

Clarified nectar	Chitosan acetate				Chitosan lactate			
	Acidity (Percentage w/w citric acid)	Soluble solids (ºBrix)	pH	Clarification yield (%)	Acidity (Percentage w/w citric acid)	Soluble solids (ºBrix)	pH	Clarification yield (%)
1	0.45 (0.14)	16.00 (0.00)	3.60 (0.50)	38.57	0.36 (0.00)	15.33 (0.26)	3.80 (0.00)	41.57
2	0.36 (0.00)	15.83 (0.28)	3.90 (0.00)	27.58	0.33 (0.03)	15.42 (0.03)	3.80 (0.00)	22.57
3	0.38 (0.02)	15.80 (0.20)	3.85 (0.02)	28.00	0.36 (0.03)	15.44 (0.02)	3.80 (0.00)	28.48
4	0.26 (0.00)	15.63 (0.02)	3.90 (0.00)	50.50	0.37 (0.00)	15.50 (0.00)	3.90 (0.00)	60.71
5	0.26 (0.00)	15.63 (0.02)	3.90 (0.00)	51.72	0.37 (0.00)	15.50 (0.00)	3.90 (0.00)	54.30
6	0.26 (0.00)	15.63 (0.02)	3.90 (0.00)	59.80	0.37 (0.00)	15.50 (0.00)	3.90 (0.00)	66.14
7	038 (0.01)	15.50 (0.00)	3.91 (0.02)	37.93	0.37 (0.00)	15.48 (0.30)	3.80 (0.00)	35.71
8	0.26 (0.00)	15.63 (0.02)	3.90 (0.00)	57.70	0.37 (0.00)	15.50 (0.00)	3.90 (0.00)	55.40
9	0.34 (0.00)	15.32 (0.01)	3.85 (0.01)	32.14	0.37 (0.00)	15.50 (0.00)	3.85 (0.01)	42.86
10	0.26 (0.00)	15.63 (0.02)	3.91 (0.01)	50.72	0.37 (0.00)	15.50 (0.00)	3.90 (0.00)	63.50
11	0.35 (0.00)	15.50 (0.00)	3.82 (0.08)	31.03	0.33 (0.33)	15.50 (0.00)	3.61 (0.00)	28.57
12	0.34 (0.00)	15.60 (0.10)	3.82 (0.05)	26.71	0.34 (0.00)	15.50 (0.00)	3.61 (0.00)	35.71
13	0.35 (0.03)	15.46 (0.05)	3.86 (0.10)	41.38	0.45 (0.21)	15.43 (0.05)	3.62 (0.00)	57.14
Mean (Standard deviation); n=3								

Table 3: Physicochemical parameters of clarified orange nectars and clarification yield of chitosan acid salts.

Influence of chitosan acid salts on the clarification yield: Table 3 shows the yields of the clarification process for each of the combinations of time and concentration depending on the type of chitosan acid salt. The chitosan lactate, as coagulant, was more effective than the chitosan acetate for obtaining a greater volume of clarified nectar, since it was achieved the highest yield (66.14%) at 90 min and concentration of 1 g/L.

A possible explanation for the greater effectiveness of chitosan lactate as a coagulant may be related to the dissociation constants (Ka) of lactic acid and acetic acid reported as 1.38×10^{-4} and 1.75×10^{-5} at 25°C, respectively. Lactate ion has a dipole bond because of its hydroxyl group, which gives a higher electrical charge density and thus has a Ka value greater than the acetate ion. Lactate ion attracts water molecules with higher ionic strength, which results in increased contact areas with the colloidal particles in suspension [30], that are in juices and nectars. Thus, lactate ion exerts an electrostatic force higher on the electrical charges of these particles, decreasing the energy barrier that prevents their agglomeration and promoting the formation of flocs [31].

In order to understand the process of clarification, some studies have been conducted to analyze the interaction between chitosan, proteins and carbohydrates. Boeris et al. reported an electrostatic interaction between chitosan and pepsin respect to the pH dependence, while Marudova et al. studied the interaction between chitosan and pectin, finding that chitosan binding capacity at pH 5.6 depends on the esterification of pectin [32,33].

Table 4 shows the significance of analysis of variance of the regression and the estimated coefficients for the yield as response variable. It is observed that the model is significant ($p \leq 0.05$) with a confidence level of 95.0%. The R2 indicated that the adjusted model explains the 94.98 and 92.90% of the variability in the yield for chitosan acetate and chitosan lactate, respectively. The lack of fit test was not significant ($p>0.05$). The residue analysis did not show atypical observations and the standardized residuals followed a normal distribution with mean zero and standard deviation equal to one.

Source	**p-value**	
	Chitosan acetate	**Chitosan lactate**
Model	0.0002	0.0007
A	0.0298	0.6383
B	0.0983	0.3693
AB	0.8908	0.5839
A2	0.0048	0.0038
B2	<0.0001	0.0004
R2	0.9498	0.929
Lack of fit	0.7962	0.5664

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A: chitosan acid salt concentration; B: clarification time

Table 4: Analysis of variance for the clarification yield with chitosan acid salts.

For the clarification process with chitosan acetate, it can be observed that the concentration (A), quadratic concentration (A^2) and quadratic clarification time (B^2) were significant factors ($p \leq 0.05$), while the clarification time (B) and interaction between concentrations and time (AB) resulted non-significant factors. When the chitosan lactate was used, only the quadratic concentration (A^2) and quadratic clarification time (B^2) resulted significant factors ($p \leq 0.05$). The models´ equations for the clarification process with chitosan acetate [Equation 2] and chitosan lactate [Equation 3] were as follows:

$$\text{Yield } (\%) = 59.98 + 4.8A + 3.37B + 0.31AB - 10.56A^2 - 20.63B^2 \quad \text{[Equation 2]}$$

$$\text{Yield } (\%) = 53.07 + 0.83A - 1.62B - 1.18AB - 10.54A^2 - 15.48B^2 \quad \text{[Equation 3]}$$

In both cases, the analysis of the models´ coefficients showed that the quadratic clarification time (B^2) had the greater influence on yield, followed by the quadratic concentration (A^2) terms, which explains the fact that the at the highest clarification time and concentration values, it was obtained the lower yields (Figure 1).

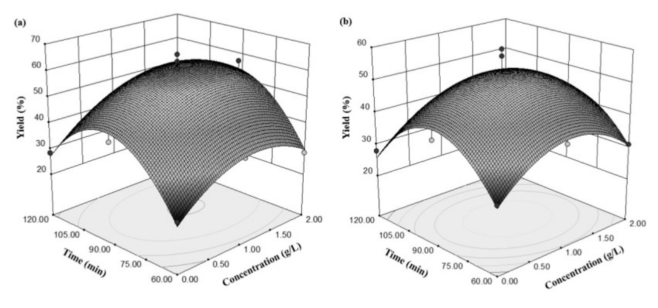

Figure 1: Influence of agitation time, chitosan acetate (a) and chitosan lactate (b) concentrations on the clarification yields of orange nectars.

Sensory evaluation: Figure 2 shows the quantitative descriptive profiles of orange nectars after 90 min of clarification with 1 g/L and 2 g/L of chitosan acetate and chitosan lactate. Theses variants were selected considering the results from the models above explained. Orange nectar had a fruity and acidic taste. As noted, there was no significant influence ($p>0.05$) of the type and concentration of chitosan acid salt on most tested sensory descriptors, but for the case of using chitosan acetate, the overall acceptance assigned by the judges was lower when a concentration of 2 g/L was used.

In both cases, nectars clarified with chitosan acetate and chitosan lactate, the largest difference was observed for the case of turbidity and showed higher values when 1 g/L of salt was used.

The overall quality assigned by the judges to orange nectar clarified with chitosan lactate was higher than for products clarified with chitosan acetate.

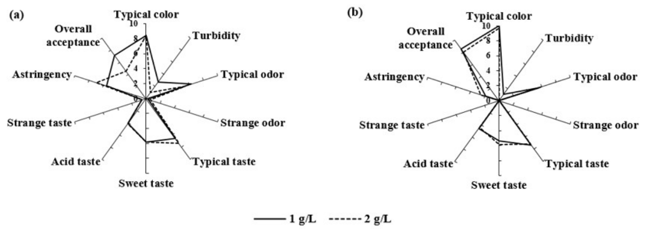

Figure 2: Quantitative descriptive profiles for orange nectars clarified during 90 min with chitosan acetate (a) and chitosan lactate (b) at different concentrations.

Conclusion

No significant effect of the salt type and concentration or clarification time on the physicochemical parameters of nectars were observed. Both chitosan acid salts could be used as clarifying agents, although chitosan lactate at 1 g/L was more effective, obtaining the highest yield (66.14%) to a time of 90 min. In general, there was no significant influence of the type and concentration of chitosan acid salt on the sensory attributes of the products and nectar clarified with chitosan lactate showed the higher overall quality. Although the quality of clarified nectars was acceptable, the optimization of the clarification process and the assessment of technical and economic feasibility of using chitosan acid salts as clarifying agents of juices and nectars are still needed for a successful application at industrial scale.

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