ORGANIC APPLE JUICE CLARIFICATION: PHYSICOCHEMICAL AND SENSORY EVALUATION

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n the Brazilian market of non-alcoholic beverages, apple juice is found clarified or turbid. In addition to its consumption as juice itself and nectar, it can also be used in soft drinks formulation. The aim of this work was to clarify organic apple juice of Eva variety using enzymatic complex (pectinases), gelatin and bentonite (phase 1) and to compare physicochemical and sensorial clarified and unclarified Eva apple juices (phase 2). The experimental design was completely randomized. In phase 1, the tests were performed on bench scale, adopting a factorial schedule and in phase 2, the tests were performed on a pilot plant scale. Parameters such as soluble solids, titratable acidity, ratio, pH, reducing sugars, total reducing sugars, non-reducing sugars, phenolic compounds, turbidity and color were evaluated for the physicochemical characterization of juices. The sensory analysis of juices were performed by affective test (hedonic scale). In phase 1, the best juice clarification results were obtained using the enzymatic complex, gelatin at concentrations of 8, 10 and 12 g.100 L^{-1} and bentonite at 50 g.100 L^{-1}, with final juice filtration in paper of 28 μm pore. In phase 2, the clarification process interfered on physicochemical characteristics of the juice for all analyzed parameters, except for total reducing sugars and non-reducing sugars. Despite differences in physicochemical composition, the panel of tasters showed equal acceptability between clarified and unclarified juices.

KEY WORDS: NON-ALCOHOLIC BEVERAGE; PECTINASES; MALUS DOMESTICA, BENTONITE, ENZYMATIC COMPLEX.

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1 INTRODUCTION

The concern of Brazilians with health and welfare increases day by day. In terms of food, in addition to careful reading of labels on food and beverage packaging, a significant share of the population is concerned about the origin of food and means of production. Therefore, the interest in organic foods and beverages is growing; the increase in the number of organic farmers as well as the area of production is an evidence of this development. According to Taques (2015), in 2014 there were 10,194 organic farmers in Brazil, representing an increase of 51% over the previous year. The production area reached 750,000 hectares in the Southeast (44%), North (21%), Northeast (16%), Midwest (14%) and South (5%). These data, from Brazilian Ministry of Agriculture, Livestock and Supply, refer to registered farmers as organic, without considering the small organic producers of fruits and vegetables that perform this type of activity in the cities. Thus, it is likely that data showed above is actually larger.

Approximately 15% of the conventional apple production is rejected because it does not have the quality standard demanded by the consumer. According to organic producers of São Paulo State, for organic apple production this rejection is around 30%. The rejected product is usually processed as juice (WOSIACKI and NOGUEIRA, 2010).

The industrial production of organic or conventional apple juice is established by normative instruction n. 1 of January 7, 2000, which approves the technical regulations for Identity and Quality Standards of fruit pulp and juices. This normative also defines apple juice as "unfermented and undiluted beverage obtained from the edible part of the apple (*Pyrus malus*, L.), through an appropriate technological process "(BRASIL, 2000).

Some consumers accept apple juice with turbidity, a fact that can be observed in organic and cultural fairs of São Paulo State cities. This kind of beverage is manufactured by small organic producers, who sell their handmade production to consumers looking for high quality products. However, most consumers prefer the transparent beverage without turbidity and sediment at the bottom of the packaging (WOSIACKI and NOGUEIRA, 2010). In order to satisfy this last group, the clarification process is necessary.

Clarification is a slow and discontinuous process used to avoid turbidity and sediment in the final product. It is performed through the addition of pectinolytic enzymes and clarifying agents (ARAYA-FARIAS et al., 2008). The turbidity of fresh processed fruit juices occurs due to pectin particles as well as cellulose, hemicellulose, lignin, starch, proteins, tannins, etc. (ALMEIDA et al., 2007). Dark pigments are also produced in apple juice due to the presence of the enzyme polyphenoloxidase, substrates of this enzyme, phenols, and precursors of the pigments, which are industrially removed by clarification agents such as bentonite and gelatin, used individually or in combination (ONSEKIZOGLU et al., 2010).

Kempka et al. (2013) evaluated the clarification of apple juice of two cultivars, Gala and Fuji, using different concentrations of a commercial enzymatic complex and a hydrolyzed collagen, under different temperatures. They concluded that apple juice clarification was efficiency with the application of the hydrolyzed collagen together with the enzymatic complex; however, they noted that further studies needs to be performed in order to optimize the process of apple juice clarification using enzymatic treatment and hydrolyzed collagen.

The aim of this research was to clarify organic apple juice of the variety Eva , using enzymatic complexes (pectinases), gelatin and bentonite in different concentrations (phase 1), and to evaluate changes in physicochemical characteristics and sensorial acceptability of clarified and unclarified apple juice (phase 2).

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL PLANNING

The research was accomplished in two phases: clarification of apple juice at bench scale (Phase 1) and pilot plant scale (Phase 2). In phase 1, the experimental design was in a factorial scheme, where gelatin was the first factor and bentonite the second. In phase 2, the design was completely randomized. The tests in phases 1 and 2 were performed with two replicates. The chemical analyzes of the apple juices were repeated twice for each sample. The results obtained in phase 1 allowed determining the clarification parameters of the apple juice of phase 2.

2.2. PROCESSING

2.2.1. PHASE 1 – BENCH SCALE

For juice extraction, organic apples of the variety Eva, from a farm located in the surroundings of Botucatu, São Paulo State, were defrosted, cut into eight parts, wrapped in fine mesh synthetic fabric (voil) and manually pressed in a 20 litres stainless steel Ranazzi screw press, made in Brazil.

The enzymatic clarification was performed with Novozymes pectinase complex (Novozym 33095), provided by the company LNF Latin American, specific for clarification of apple juice. The dosage used was that recommended by the manufacturer: 3 mL.100 L^{-1}. The enzymatic treatment was carried out at 45 °C for one hour, followed by three hours resting at room temperature (20 °C). Thereafter, clarification blends were prepared with gelatin at the concentrations of 0, 2, 4, 6, 8, 10 and 12 g. 100 L^{-1} of apple juice. The juice was kept at 5 °C for one hour. Then, the apple juice was clarified with bentonite at the concentrations of 0, 30, 40 and 50 g. 100 L^{-1}. The juice rested for two hours at 5 °C. At the end of the clarification process, a 20 mL sample of the juice was taken to determine turbidity without filtration. The remaining juice was then filtered on paper of 28 µm pore and analyzed for turbidity (CARVALHO et al, 2011). Figure 1 shows the flowchart of operations used in phase 1.

2.2.2. PHASE 2 – PILOT PLANT SCALE

For juice extraction, the apples were defrosted, crushed in a stainless steel hammer mill, Mecamau, model M037, made in Brazil, with a 18 mm hole sieve. The crushed apple was wrapped in a fine mesh synthetic fabric and smashed at 108 bar for 2 minutes, in a 50 litres AGM automatic hydraulic press, made in Brazil.

For clarification, the juice was treated with the same enzymatic complex used in Phase 1 in the proportion of 3 mL.100 L^{-1} and kept at room temperature for 3 hours in 4 litre polypropylene beaker. After this period, gelatin (4 g. 100 L^{-1}) was added to the juice and the mixture was kept for 1 hour at 5-8 °C. Then, bentonite (30 g. 100 L^{-1}) was added to the juice and kept for 2 hours at the same temperature. The supernatant was transferred to 1litre beakers and kept at room temperature for 3 hours for sediment settling. The supernatant was transferred to 500 ml glass bottles, using a peristaltic pump Cole Parmer, Masterflex L/S, made in Malaysia, and bottles were frozen in a freezer (-15 ° C) (CARVALHO et al, 2011).

Unclarified juice (control) was extracted by hammer mill and hydraulic press, added to 1 litre beakers and kept at room temperature for 3 hours, for sediment settling of the insoluble solids. It was then bottled and frozen, as previously described.Figure 2 shows the flowchart of phase 2 operations.

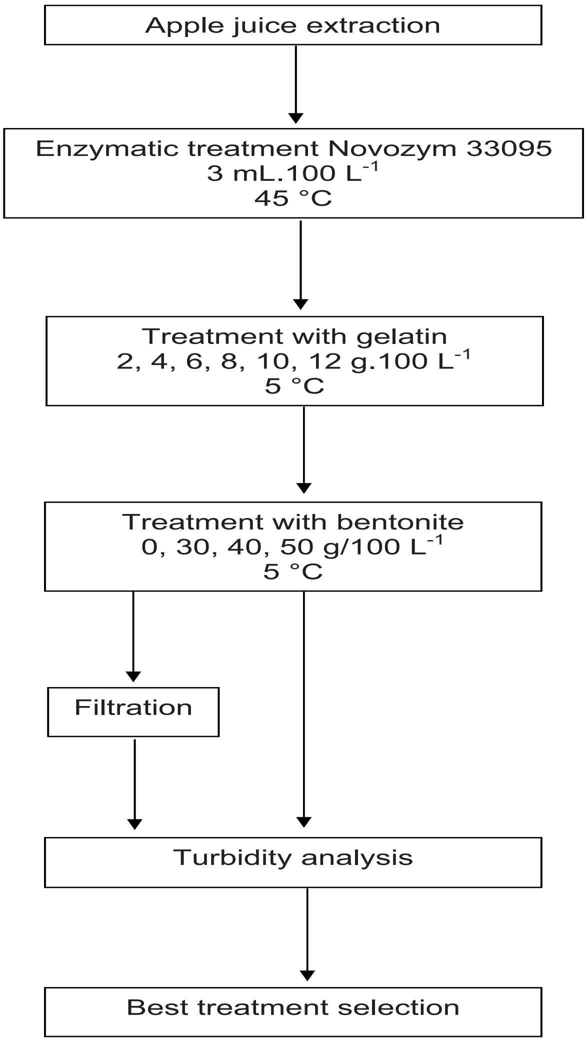

FIGURE 1. FLOWCHART OF PHASE 1 OPERATIONS.

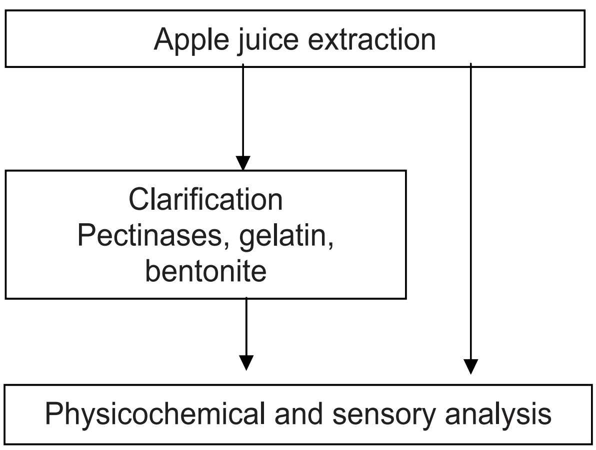

FIGURE 2. FLOWCHART OF PHASE 2 OPERATIONS.

2.3. PHYSICOCHEMICAL ANALYSES (PHASE 2)

The methodologies for physicochemical analyses of clarified and unclarified apple juice are described in Brasil (2005).

Soluble solids (SS), analyzed by digital refractometer Reichert, model r^{2}I300, made in USA. Results are expressed in °Brix;

Tritable acidity (TA), analyzed by tritation with sodium hydroxide 0,1 M. Results are expressed in grams of malic acid per 100 mL of juice;

Ratio, calculated by the rate between soluble solid and total acidity;

pH, analyzed in Tecnal pH meter, model TEC-5, made in Brazil;

Reducing sugars (RS), analyzed by tritation with Fehling. Results are expressed in grams of glucose per 100 mL of juice (COPERSUCAR, 1978);

Total reducing sugars (TRS), as previous item, with sucrose hydrolysis performed by using hot chloridric acid solution (COPERSUCAR, 1978);

Non-reducing sugars in sucrose (NRS), calculated by: **(TRS – RS)*0,95**;

Phenolic compounds (FC), analyzed by Folin-Ciocalteau methodology. Results are expressed in mg of galic acid per litre of juice (OIV, 2009);

Turbidity, analyzed by turbidimeter Hach, model 2100N, made in USA. Results are expressed in NTU;

Color, analyzed by spectrophotometer Biochron, model Libra S60, made in England. After filtering in paper of 28 μm pore, the sample was analyzed in 440 and 520 nanometers wavelength.

2.4. SENSORY ANALYSIS (PHASE 2)

The sensory analysis of clarified and unclarified apple juices was performed by nine points hedonic scale, using scores ranging from 1 (I highly disliked) to 9 (I liked it very much) in order to classify the preference. Tasters evaluated two samples simultaneously, giving scores for the attributes color, transparency, aroma, flavor and overall assessment. The juice samples were tasted at 5-8 °C in glass cups under white light, coded with randomized three numbers. Employees and students of São Paulo State University (Unesp), males and females, ranging from 17 to 60 years old, composed the sensory panel (BEHRENS, 2011).

2.5. STATISTICAL ANALYSIS

The apple juice clarification data from phase 1 were evaluated by variance analysis and followed by Tukey's test. For clarified and unclarified apple juices from phase 2, data from the physicochemical analyzes, following normal distribution, were analyzed by Student's t-test. The non-parametric sensory analysis data were analyzed by the Mann-Whitney test.

Minitab 16 software was used to perform the statistical analyzes and it was considered significant when $p < 0.05$, for all tests of the two phases (MINITAB 16, 2010)

2.6. RESEARCH ETHICS COMMITTEE

Due to the participation of humans beings, this research was registered in the Brazilian National System of Research Ethics (SISNEP). The research was approved by Research Ethics Committee of Botucatu Medical School – UNESP. CAAE: 48272215.1.0000.5411. Receipt number: 079654/2015

3. RESULTS AND DISCUSSION

3.1. PHASE 1

The turbidity results in Table 1 corresponds to the clarification of apple juice using the enzymatic complex, gelatin and bentonite, followed by filtration. The best results ($p < 0.01$) were obtained with bentonite (50 g.100 L^{-1} of juice), associated to gelatin (8 to 12 g. L^{-1}). The worst results were obtained in the treatments with low concentrations of gelatin and absence of bentonite. These results show that in gelatin treatments with bentonite the flakes formed were sufficiently large to be retained by the pores (0.28 μm) of the paper filter, whereas in the treatments that did not use the bentonite, a part of the gelatin in the colloidal state passed throught the filter barrier.

TABLE 1. TURBIDITY MEAN DATA, IN NTU, OF CLARIFIED APPLE JUICE WITH PECTINASES, GELATIN AND BENTONITE, WITH FILTRATION.

Gelatin	Bentonite (g.100 L^{-1})				Means
(g.100 L^{-1})	0	30	40	50	
2	52,7 ABa	38,4 Ab	36,6 Ab	9,2 Ac	34,2 A
4	63,4 Aa	9,4 Cb	13,3 Bb	4,9 Ab	22,8 B
6	46,7 Ba	5,5 Cb	11,6 Bb	2,9 Ab	16,6 C
8	26,8 Ca	7,8 Cb	13,2 Bb	2,5 Ab	12,6 CD
10	21,9 Ca	8,9 Cb	12,4 Bab	3,1 Ab	11,6 D
12	8,7 Db	22,5 Ba	10,7 Bab	2,8 Ab	11,2 D
Means	36,7 a	15,4 b	16,3 b	4,2 c	

Means followed by equal letters and uppercase in the column and lowercase letters in the line do not differ by the Tukey test (5% significance).

The results of Table 2 express the clarification of apple juice with pectinases enzymes, gelatin and bentonite, but without filtration in the end of process. The best results ($p <0.01$) were obtained with bentonite (30 and 50 g.100 L^{-1}), associated with the lowest gelatin concentrations, 2 to 10 g.100 L^{-1}. The worst results were obtained in the highest gelatin concentration and without bentonite. In these cases, the high turbidity is due to the remaining gelatin in colloidal state in the apple juice. The function of bentonite is just to remove the excess of gelatin remaining in the juice.

Benitez and Lozano (2007) working with apple juice clarification, using concentrations (%) of bentonite (0,16; 0,32; 0,48; 0,64) and gelatin (0,016; 0,032; 0,048; 0,064), found results "at concentrations of bentonite >0.32 juices was practically clarified (NTU≈ 0) at any of the assayed gelatin concentrations greater than 0.02%. Moreover, at lower bentonite concentration (<0.32%) the amount of gelatin necessary to drastically reduce turbidity was found to be 0.032%". Although Benitez and Lozano (2007) work had been performed using different additives concentrations, it shows that the complex composed by bentonite and gelatin is well succeded in apple juice clarification.

Comparing the turbidity values of Tables 1 and 2, the filtered juices had the lowest values of turbidity. However, since this research is focused on using organic apple from small farmers, simplifying the process and reducing the cost of production, the authors decided to accomplish the clarification of the juice without the final filtration, in phase 2.

3.2. PHASE 2

The clarified juice had soluble solids content higher than the control juice (Table 3). Probably, this is due to the presence of the polygalacturonase and pectin lyase enzymes present in the enzymatic complex used to clarify the juice, because the action of these enzymes on pectin results in the release of galacturonic acid (DAMODARAN et al., 2010). As with sugars, organic acids also contribute to the soluble solids content of fruit juices (QUEIROZ; MENEZES, 2010).

The release of galacturonic acid units may have been responsible for the reduction of pH and increase of acidity in clarified juice. This process of acidification also occurs by the participation of the pectinamethylesterase enzyme that reduces the tax of esterification or methoxylation of pectin, restoring the acidic character of the molecule (DAMODARAN et al., 2010). The lower ratio value measured in clarified juice is mainly due to its higher acidity.

As the concentration of total reducing sugars is greater than reducing sugars, it is an indicative of the presence of non-reducing sugars in the form of sucrose in both juices. The non-reducing sugars express the concentration of sucrose originally present in apple juice. On the other hand, the clarified juice had higher reducing sugars content. Pectin presents pentoses (rhamnose, arabinose, xylose) in its composition, being the pentoses a reducing sugars and its release in the juice due to pectinases action, it favors the increase of the reducing power of the clarified apple juice.

TABLE 2. TURBIDITY MEAN DATA, IN NTU, OF CLARIFIED APPLE JUICE WITHOUT FILTRATION.

Gelatin (g.100 L^{-1})	0	30	40	50	Means
2	68,7 Ba	17 Ba	29,4 Ba	7,3 Aa	30,6 B
4	76,9 Ba	12,3 Ba	61,5 Ba	5,7 Aa	39,1 B
6	92,9 Ba	15,4 Ba	24,0 Ba	11,0 Aa	35,8 B
8	110,0 ABa	28,5 ABab	47,9 Bab	12,7 Ab	49,8 B
10	139,5 ABa	36,6 ABb	39,9 Bb	14,8 Ab	57,7 B
12	191,5 Aa	73,3 Abc	150,8 Aab	22,4 Ac	109,5 A
Means	113,2 a	30,5 c	58,9 b	12,3 c	

Means followed by equal letters and uppercase in the column and lowercase letters in the line do not differ by the Tukey test (5% significance).

TABLE 3. PHYSICOCHEMICAL ANALYSES OF APPLE JUICE WITH AND WITHOUT CLARIFICATION.

	Non-clarified juice (control)	Clarified juice
Soluble solids (Brix)	13,2 ± 0,49 b	14,1 ± 0,18 a
pH	4,3 ± 0,06 a	3,9 ± 0,02 b
Tritable acidity (%)	0,2 ± 0,01 b	0,3 ± 0,01 a
Ratio	56,9 ± 3,70 a	45,8 ± 1,43 b
Reducing sugars (%)	8,7 ± 0,48 b	9,2 ± 0,22 a
Total reducing sugars (%)	10,3 ± 0,28 a	10,8 ± 0,30 a
Non-reducing sugars (%)	1,6 ± 0,44a	1,5 ± 0,24a
Phenolic compounds (mg EAG/L)	386,0 ± 5 a	330,0 ± 3 b
Turbidity (NTU)	52,5 ± 3,93 a	13,9 ± 10,02 b
Color (absorbance 440 e 520 nm)	1,5 ± 0,02 a	1,1 ± 0,03 b

Means followed by equal letters in the line do not differ by Student's t-test ($p<0.05$).

It is probable that during the clarification of the juice, phenolic compounds (quinones) have reacted with sulfhydryl and amine groups of the proteins (DAMODARAN et al., 2010) and forming irreversible and insoluble complexes that are separated from the juice by decantation, which explains the lower content of phenolic compounds of clarified juice.

The lower turbidity of the clarified juice takes place from the solubilization of pectin by pectinases (polygalacturonase and pectin lyase) that reduce the size of the molecule. This occurs similarly to the hydrolysis of the starch and by the formation of insoluble complex with the proteins and polyphenols of the juice that are separated by decantation (DAMODARAN et al., 2010 and WOSIACKI, NOGUEIRA, 2010).

The color intensity of clarified juice may have been reduced due to the removal of pigments by the clarification process. From the three types of important molecules involved in the clarification of apple juice (proteins, pectins and polyphenols), the latter are associated with juice color, and their oxidation results in brown compounds, as reported by Wosiacki and Nogueira (2010) and Damodaran et al. (2010).

Wosiacki and Nogueira (2010) published physicochemical analyses data of apple juices produced from 152 fruit cultivars (commercial, pollinators and advanced selections) during the harvests of 1982 and 2007. Except for non-reducing sugars and turbidity analyses, which were not made by these authors, all others analyses performed in this research (soluble solids, pH, titratable acidity, ratio, reducing sugars, total reducing sugars, phenolic compounds and color) remained within the range of variation presented by them.

The results shown in Table 3 are in accordance to those presented by Nogueira et al. (2006), who chemically analyzed apple juice from the commercial varieties Belgolden, Fredhough, Melrose and Sansa. Kempka et al. (2013) studied the apple juice of the commercial varieties Fuji and Gala. The results of these researches were similar to those presented in Table 3. Therefore, the physicochemical characteristics of the organic apple juice of the variety Eva are similar to other commercial varieties produced in Brazil.

The results from sensory analysis suggest the same acceptability of clarified and unclarified juices by the panel of tasters, for all evaluated attributes. These results indicate that the chemical differences (soluble solids, pH, titratable acidity, ratio, reducing sugars and phenolic compounds) and physical differences (color and turbidity) between the two juices were not perceived by the panel of tasters; Or if they were, the tasters attributed similar acceptability scores to the assessed attributes of both beverages. Except for the aroma attribute that had score 6 (I liked it slightly), the other attributes (color, transparency, flavor and overall assessment) received score 7 (I liked it regularly). Whether the work were accomplished with fresh apples, the attribute scores would probably be higher. The apples used in the present research were harvested between December 2013 and January 2014, kept in a cold chamber (4-8 °C) for approximately three months and frozen for another five months, when they were processed.

TABLE 4. SENSORY ANALYSIS OF APPLE JUICES WITH AND WITHOUT CLARIFICATION BY HEDONIC SCALE TEST.

	Unclarified juice (Control)	Clarified juice
Color	7 a	7 a
Transparency	7 a	7 a
Falvor	7 a	7 a
Aroma	6 a	6 a
Overall assessment	7 a	7 a

Means followed by equal letters in the line do not differ by Mann-Whitney test (p<0,05).

As this research was developed according to the interests of small producers of organic apple of the variety Eva, the results tend to the processing of unclarified integral juice, which does not require the use of enzyme complex, gelatin, bentonite, and filter. This implies in a lower cost of production and simplifies the process of craft production of the beverage. The authors of the present work suggest that the handmade turbid apple juice is locally marketed, exploiting a market share of Brazilian society: organic products. In addition, the turbid juice can be frozen in polyethylene packages and stored in a freezer to be used in the production of nectar or apple soda, considering the local market demand.

According to exposed, new research projects should be performed to technically enable the production of non-alcoholic beverages based on organic apple of the Eva variety.

4 CONCLUSION

The best clarification results of organic apple juice of the Eva variety are obtained by using the enzymatic complex Novozym 33095 at the concentration recommended by the manufacturer, gelatin at concentrations of 8, 10 and 12 g. 100 L^{-1} and bentonite at 50 g. 100 L^{-1}, followed by filtration.

The clarification of organic apple juice of the Eva variety with pectinases, gelatin and bentonite alters its physicochemical characteristics, but does not interfere in its acceptability.

To small farmers, the production of unclarified apple juice is recommended due to the easiness of obtaining the final product and reduction of the production costs, as this beverage has the same acceptability of clarified juice.

RESUMO

CLARIFICAÇÃO DE SUCO DE MAÇÃ ORGÂNICA: AVALIAÇÃO FÍSICO-QUÍMICA E SENSORIAL

No Mercado brasileiro de bebidas não alcoólicas, o suco de maç*ã é encontrado clarificado ou turvo.* Al*ém de ser consumido como suco, propriamente dito, e* néctar, também poder ser utilizado na formulaç*ão de refrigerantes.* O objetivo do presente trabalho foi clarificar suco de maçã orgânico da variedade Eva, utilizando complexo enzimático (pectinases), gelatina e bentonita (fase 1) e comparar físico-química e sensorialmente sucos de maçã clarificados e não clarificados (fase 2). O delineamento experimental foi inteiramente casualizado, sendo que na fase 1, os testes foram realizados em escala de bancada, adotando um esquema fatorial e na fase 2, os ensaios foram realizados em escala piloto. Para a caracterização físico-química dos sucos avaliou-se os sólidos solúveis, acidez titulável, ratio, pH, açúcares redutores, açúcares redutores totais, açúcares não redutores, compostos fenólicos, turbidez e cor. A análise sensorial dos sucos foi realizada por teste de afetivo (escala hedônica). Na fase 1, os melhores resultados de clarificação de suco ocorreram com a utilização do complexo enzimático, gelatina nas concentrações de 8, 10 e 12 g.100 L-1 e bentonita a 50 g.100 L-1, com filtração final do suco em papel com poro de 28 μm. Na fase 2, o processo de clarificação alterou as características físico-químicas do suco para todos os parâmetros analisados, exceção feita aos açúcares redutores totais e açúcares não redutores. Apesar das diferenças na composição físico-química, o painel de provadores mostrou igual aceitabilidade entre os sucos clarificados e não clarificados.

PALAVRAS-CHAVE: BEBIDA NÃO ALCOÓLICA; PECTINASES; MALUS DOMESTICA, BENTONITA, COMPLEXO ENZIMÁTICO.

REFERENCES

1. ABDULLAH, A.G. L.; SULAIMAN, N.M.; AROUA, M.K.; MEGAT MOHD NOOR, M.J. Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. **Journal of Food Engineering**, v. 81, p. 65–71, 2007.

2. ARAYA-FARIAS, M.; MONDOR, M.; LAMARCHE, F.; TAJCHAKAVIT, S.; MAKHLOUF, J. Clarification of apple juice by electroflotation. **Innovative Food Science and Emerging Technologies**, v. 9, p.320–327, 2008.

3. BEHRENS, J. Análise sensorial de bebidas. In: VENTURINI FILHO, W. G. (Coord.). **Indústria de bebidas**: inovação, gestão e produção. São Paulo: Blucher, 2011. cap. 9, p. 183-213.

4. BENITEZ, E. I.; LOZANO, J. E.. Effect of gelatin on apple juice turbidity. **Latin American Applied Research**. Bahía Blanca , v. 37, n. 4, p. 261-266, 2007 . Available in: <http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S0327-07932007000400006&lng=es&nrm=iso>. Accessed Aug. 17 2017.

5. BRASIL. Instrução normativa n. 01, de 07 de janeiro de 2000. Aprova os Regulamentos Técnicos para Fixação dos Padrões de Identidade e Qualidade para polpa das seguintes frutas: acerola, cacau, cupuaçu, graviola, açaí, maracujá, caju, manga, goiaba, pitanga, uva, mamão, cajá, melão, mangaba, e para suco das seguintes frutas: maracujá, caju, caju alto teor de polpa, caju clarificado ou cajuína, abacaxi, uva, pêra, maçã, limão, lima ácida e laranja. Disponível em: <www2.agricultura.rs.gov.br/uploads/126989581629.03_enol_in_1_00_mapa.doc+&cd=1&hl=en&ct=clnk&gl=br> Acesso em: 01 mar. 2016.

6. BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. **Métodos físico-químicos para análise de alimentos**. Brasília: Ministério da Saúde, 2005. 1018p.

7. CARVALHO, J. R.; SILVA, K. M.; BRAGA, C. M.; ALBERTI, A.; WOSIACKI, G.; NOGUEIRA, A. Efeito da clarificação com gelatina no teor de compostos fenolicos e na atividade antioxidante de fermentados de maçãs. **Brazilian Journal of Food Technology**, Campinas, v. 14, p. 41-49, 2011.

8. COPERSUCAR. **Controle químico da fabricação de açúcar**. Cooperativa Central dos Produtores de Açúcar e Álcool do Estado de São Paulo, 1978. 127 p.

9. DAMODARAN, S.; PARKIN K. L.; FENNEMA, O. R. **Química de alimentos de Fennema**. Porto Alegre: Artmed, 2010. 900 p.

10. KEMPKA, A. P.; PRESTES, R. C.; ALVIERO, T. Clarificação de suco de maçã de dois cultivares utilizando tratamento enzimático e colágeno hidrolisado**. Revista Brasileira de Produtos Agroindustriais**, Campina Grande, v.15, n.2, p.137-146, 2013.

11. MINITAB 16 Statistical Software, Minitab Inc., State College, PA, USA, 2010.

12. NOGUEIRA, A. et al. Características físico-químicas e sensoriais de suco de maçã clarificado e fermentado. **Publicatio UEPG**: Ciências Exatas e da Terra, Agrárias e Engenharias. Ponta Grossa, v. 12, n. 3, p. 15-23, 2006.

13. OIV - Organisation Internationale de la Vigne et du Vin.C**ompendium of international methods of wine and must.** v. 1. 2009 ed. Disponível em: <http://news.reseau-concept.net/images/oiv_es/Client/Compendium_2009_Vol1_EN.pdf>. Acesso em: 18 jan. 2011.

14. O'NEIL, M. J. et al. **The Merck index**. Whitehouse Station: Merck Research Laboratories, 2006. 1756 p.

15. ONSEKIZOGLU, P.; BAHCECI, K. S.; ACAR, M. J. Clarification and the concentration of apple juice using membrane processes: A comparative quality assessment. **Journal of Membrane Science**, v. 352, p.160–165, 2010.

16. PINELO, M.; ZEUNER, B.; MEYER, A. S. Juice clarification by protease and pectinase treatments indicates new roles of pectin and protein in cherry juice turbidity. **Food and Bioproducts Processing**, v. 88, p. 259–265, 2010.

17. QUEIROZ, E. C.; MENEZES, H. C. Suco de laranja. In: VENTURINI FILHO, W. G. (Coord.). **Bebidas não alcoólicas**: ciência e tecnologia. São Paulo: Blucher, 2010. v. 2, cap. 14, p. 243-267.

18. TAQUES, F. Você já pensou em consumir algum alimento orgânico? **Diário da Serra**, Botucatu, ano 24, n. 5736, p. a2. 10 jun. 2015.

19. WOSIACKI, G.; NOGUEIRA, A. Suco de maçã. In: VENTURINI FILHO, W. G. (Coord.). **Bebidas não alcoólicas**: ciência e tecnologia. São Paulo: Blucher, 2010. v. 2, cap. 15, p. 269-302.