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Optimization of inulin:Oligofructose proportion and non-thermal processing to enhance microbiological and sensory properties of fiber-enriched strawberry juice

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ABSTRACT

Hurdle effect of vanillin concentration (0–1.25 mg/mL) and ultrasound time (0–30 min) with juice formulation (inulin:oligofructose proportion) on microbiological and sensory quality of strawberry juice after 14 days of storage at 5 °C were studied. Response surface methodology (RSM) and Box-Behnken design were used to find the conditions that simultaneously optimize: mesophilic aerobic bacteria, *Enterobacteriaceae* and total coliform and yeasts and molds (as microbiological indices) and overall visual quality (OVQ), typical strawberry odor and off-odor (as sensory attributes). Individual optimization of each response was carried out and compared with a simultaneous optimization using the Desirability function. Determination coefficients (R^2) for the second-order models adjusted by RSM were above 96%. Microbiological indices were significantly affected by vanillin whatever ultrasound time and fibers proportion were assayed. Vanillin and ultrasound resulted critical factors for all sensory attributes studied. Fibers proportion did not modify microbiological and sensory indices. Conditions that simultaneously optimize all responses were: 1.25 mg/mL of vanillin, 7.5 min of ultrasound time and 5:3 of inulin:oligofructose proportion. These results were validated and it was demonstrated that juice treated under optimal conditions resulted with enhanced microbial and sensory attributes.

1. Introduction

During last years, consumers, more aware of the impact of food on their health, are demanding safer, fresher and healthier food products (Duan & Zhao, 2009; Keenan, Brunton, Butler, Wouters, & Gormley, 2011). In this context, fruit juices are gaining popularity as they also offer ease of use. In particular, strawberry juice is one of the most consumed juices since it has an excellent acceptability and is considered a rich source of antioxidant compounds (Oszmiański & Wojdyło, 2009).

One way to attend the demand of healthier food is by enriching food products with functional ingredients, such as prebiotics (Keenan et al., 2011). Prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). Many substances have been reported to deliver prebiotic effects. Among them, inulin and oligofructose have widely been reported as prebiotics as they have shown to manipulate the composition of the colonic microflora (Cardarelli, Buriti, Castro, & Saad, 2008). Inulin and oligofructose can be used for either their nutritional advantages or technological properties, but they are often applied to offer a dual benefit: an improved organoleptic quality and a better-balanced nutritional composition (Franck, 2007). The physico-chemical properties of inulin are linked to the degree of polymerization. The short-chain fraction, oligofructose, is much more soluble and sweeter than native and long-chain inulin and can contribute to improve mouthfeel because its properties are closely related to those of other sugars. Long-chain inulin is less soluble and more viscous than the native product and can act as

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texture modifier (Meyer, Bayarri, Tárrega, & Costell, 2011). The addition of such ingredients to beverages have been reported by Nazzaro, Fratianni, Sada, and Orlando (2008) who studied the symbiotic potential of carrot juice supplemented with *Lactobacillus* spp. and inulin or fructooligosaccharides to produce a functional beverage. Besides, Keenan et al. (2011) studied apple purees enriched with prebiotics and processed by thermal and high hydrostatic pressure.

On the other hand, the need to develop safer food products with 'fresh-like' characteristics, has driven the research towards non-thermal preservation technologies, as traditional thermal treatments may cause some adverse effects on the nutritional and/or organoleptic attributes of food. Non-thermal techniques have the potential to ensure safety while maintaining the fresh-like characteristics of the food product (Caminiti et al., 2011).

Sonication is a non-thermal food preservation technique, which uses ultrasound for inactivating food spoilage microorganisms and enzymes, generally at a frequency of 20–40 kHz (Char, Mitilinaki, Guerrero, & Alzamora, 2010; Ferrante, Guerrero, & Maris, 2007; Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010). Ultrasound treatments have been applied to several food products to control microbial growth in orange juice (Valero et al., 2007) and to improve quality parameters in kiwifruit and mango juices (Santhirasegaram, Razali, & Somasundram, 2013; Tomadoni, Moreira, Espinosa, & Ponce, 2015).

Another alternative widely used recently to improve safety of food is the addition of naturally occurring antimicrobials (Ferrante et al., 2007), such as essential oils, vegetable extracts, bacteriocins, among others (Char, Guerrero, & Alzamora, 2010; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2012). Numerous studies have proven the effectiveness of different natural compounds in the inactivation of microorganisms. In particular, vanillin (4-hydroxy-3-methoxybenzaldehyde), the major constituent of vanilla beans, not only has demonstrated to have antimicrobial and antioxidant properties, but also impairs pleasant flavor notes to a wide variety of products such as confectionery, beverages, and pharmaceuticals (Char, Guerrero, et al., 2010), being highly compatible with strawberry juices (Cassani, Tomadoni, Viacava, Ponce, & Moreira, 2016).

In a previous work, we have demonstrated that the application of vanillin to strawberry juices enriched with different prebiotic fibers was effective in reducing microorganisms' proliferation but introduced small sensory changes to the enriched juices (Cassani et al., 2016). An alternative to reduce this negative effect could be to apply this methodology together with ultrasound in order to achieve the same antimicrobial effect without affecting sensory quality. To the best of our knowledge, the antimicrobial effect of combining vanillin with ultrasound treatment against naturally occurring microorganisms in strawberry juices enriched with prebiotic fibers, as well as, its sensory impact just after processing and after two weeks of storage, has not been previously studied. Besides, it is of paramount importance to investigate the effect of adding different proportions of inulin and oligofructose on sensory attributes of strawberry juices and to study the interaction between the mix of prebiotics and the preservation techniques applied to strawberry juices. Therefore, this research was carried out with the aim of optimizing preservation factors (ultrasound time, concentration of vanillin extract) and formulation of strawberry juice (added fiber composition) using response surface methodology in order to simultaneously maximize microbial and sensory quality of juices after two weeks of storage.

2. Materials and methods

2.1. Juice obtaining

Strawberries (*Fragaria x ananassa* Duch, cultivar Camarosa) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. Fruits with defects were discarded while fruits with good visual quality were washed with tap water and their calyxes were removed. Then, fruits were squeezed with a commercial juice extractor and the fresh strawberry juice was collected and mixed in a glass jar. Eighteen juice portions were bottled under hygienic conditions into 350 mL polyethylene terephthalate (PET) bottles and sealed with polyethylene (PE) caps to be enriched with inulin and oligofructose and then processed by treatments. Finally, packaged strawberry juices were stored at refrigeration temperature (5 ± 1 °C) in darkness up to analysis.

2.2. Treatments and experimental design

Proportion of inulin:oligofructose as fiber added to juices, together with the operational conditions of non-thermal processing techniques (ultrasound treatment and vanillin addition) were optimized using response surface methodology.

Response surface methodology with a Box-Behnken (BB) design was used to study the influence of ultrasound time and bioactive concentration for preservation of strawberry juice with prebiotics inclusion (in different proportions). The method of least-squares regression was used to fit data to a quadratic model of the form (for each response variable):

$$Y_n = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=2, j>i}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (1)$$

Where Y_n is the predicted response (Y_1: mesophilic bacteria counts, Y_2: *Enterobacteriaceae* and total coliforms counts, Y_3: yeasts and molds counts, Y_4: overall visual quality, Y_5: typical odor, Y_6: off-odor), β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the coefficient for the interaction effect. X_i is a dimensionless coded value of the independent variable, x_i. In this study, x_1: fiber proportion (inulin:oligofructose), x_2: ultrasound time, x_3: vanillin concentration.

Three independent experiments were carried out; each one with fifteen experimental runs combining the 3 levels of each variable as shown in Table 1. At day 0 and 14, responses were measured by duplicate for each trial. Mean values were informed for each response and were considered for fitting the second-order polynomial models (Eqn. (1)).

Three extra juice samples were used as control: strawberry juice without fibers and with no treatments applied (C1), strawberry juice enriched with an inulin: oligofructose proportion of 1:3 without preservation treatments (C2) and strawberry juice with an inulin: oligofructose proportion of 3:1 without preservation treatments (C3).

Concerning the mix of inulin:oligofructose, the addition levels were established as a function of the expected real human consumption of strawberry juice, at least 200 mL/day (a portion). Coussement (1999) suggested that inulin and oligofructose concentration of 10–60 mg/mL in prebiotic foods resulting in absolute amounts of 3–8 g per portion were required to deliver health benefits. Taking into account and based on previous experiments, 3 g of prebiotics in 200 mL of juice were evaluated at three different proportions of inulin:oligofructose (1:3, 1:1 and 3:1). The ultrasound processing times selected for this study were: 0, 15 and 30 min, according to previous unpublished experiments, while

Table 1
Box Behnken experimental design matrix.

Run	Independent variables			Coded independent variables		
	x_1 Fiber proportion (inulin: oligofructose)	x_2 Ultrasound treatment (min)	x_3 Bioactive concentration (mg/mL)	X_1	X_2	X_3
1	1:3	0	0.625	−1	−1	0
2	1:3	30	0.625	−1	1	0
3	3:1	0	0.625	1	−1	0
4	3:1	30	0.625	1	1	0
5	1:1	0	0	0	−1	−1
6	1:1	0	1.250	0	−1	1
7	1:1	30	0	0	1	−1
8	1:1	30	1.250	0	1	1
9	1:3	15	0	−1	0	−1
10	3:1	15	0	1	0	−1
11	1:3	15	1.250	−1	0	1
12	3:1	15	1.250	1	0	1
13	1:1	15	0.625	0	0	0
14	1:1	15	0.625	0	0	0
15	1:1	15	0.625	0	0	0

vanillin was applied at 0, 0.625 and 1.25 mg/mL of juice. These concentrations were selected according to Cassani et al. (2016).

Inulin and oligofructose were added to each strawberry juice bottle and stirred until total dissolution. The ultrasound treatments were performed at 40 kHz frequency (power of the ultrasound waves: 180 W transmitted from bottom to above) using an ultrasonic cleaning bath (TestLab, Argentine) of 15 × 29 × 15 cm in the dark, to avoid any light interference. Temperature in the ultrasonic bath was monitored at 20 ± 1 °C. Vanillin (Firmenich SAICYF, Argentina) was applied directly into the juice samples and stirred until total dissolution.

2.3. Response variables

The impact of adding prebiotic fibers in combination with the non-thermal preservation treatments on microbiological and sensory quality of strawberry juice was simultaneously analyzed. These indices were assessed at day 0 and 14 of refrigerated storage.

2.3.1. Microbiological analysis

The microbial stability of strawberries juices was evaluated through the enumeration of total aerobic mesophilic bacteria (MES), *Enterobacteriaceae* and total coliforms (EB) and yeasts and molds (YM) populations. A 10 mL aliquot of juice from each treatment was sampled and serial dilutions (1:10) of each sample were made in peptonated water (1 mg/mL) and surface spread by duplicate. The enumeration of the microbial populations was performed according to Ponce, Agüero, Roura, del Valle, & Moreira (2008) by using the following culture media and culture conditions: mesophilic aerobic bacteria on Plate Count Agar (PCA) incubated at 35 °C for 48 h; *Enterobacteriaceae* and total coliforms in Mac Conkey agar incubated at 35 °C for 24 h; yeasts and molds on Yeast-Glucose-Chloramphenicol (YGC) medium incubated at 25 °C for 5 d. All culture mediums were purchased from Britania, Buenos Aires, Argentina. Microbial counts were expressed as log CFU/mL.

2.3.2. Sensory quality

Quantitative descriptive analysis was used to evaluate sensory attributes of strawberry juice samples at day 0 and 14. A panel comprising ten members, aged 25–50 years with sensory evaluation experience was trained and carried out the evaluation of strawberry juices. Samples labeled with 3digit code numbers were randomly provided. The attributes evaluated were: overall visual quality (OVQ), typical odor and off-odor. The intensity of the attributes evaluated was quantified on unstructured scale from 0 to 5. OVQ was scored from 0 (highly deteriorated aspect) to 5 (fresh aspect), typical odor from 0 (not detected) to 5 (fresh) and off-odor from 0 (not detected) to 5 (intense). The limit of acceptance was 2.5, indicating that score below 2.5 for any of the attributes evaluated was deemed to indicate end of shelf-life.

2.4. Simultaneous optimization and validation

A simultaneous optimization was carried out using the Desirability function (D). For this purpose, predicted values obtained from each model (Yn) were transformed to a dimensionless desirability scale dn. The desirability scale ranges from 0 to 1, where d = 0 for an unacceptable response value, and d = 1 for a completely desirable one. The individual desirability functions from the considered responses were then combined to obtain the overall desirability D, defined as the geometric average of the individual desirability. An algorithm was then applied to this function in order to determine the set of values that maximize it (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

In order to test the reliability of the simultaneous optimization, a new set of experiments using optimal operating conditions were performed. For these experiments, a juice sample was prepared according to optimal processing conditions and two types of controls were used: (1) strawberry juice sample enriched with the optimal combination of inulin and oligofructose with no treatments application; (2) strawberry juice sample without added fibers and with no treatments applied. After treatments, strawberry juice samples were stored at 5 ± 1 °C during 14 days. The microbial population counts and sensory analysis were assessed at 0 and 14 days of storage. All assays were carried out by triplicate in two independent experimental runs.

2.5. Statistical analysis

Data were analyzed using the STATISTICA 7.1 (Statsoft Inc., Tulsa, U.S.A., 2004). The statistical analysis was performed using the analysis of variance (ANOVA) including the F-ratio, which established the model global significance and the adjusted determination coefficient R^2. The Lack of Fit test was performed for each model with a 95% confidence level. In addition, experimental and predicted values for each dependent variable were compared. The significant factors affecting each dependent variable were selected according to the Student t-test establishing a 95% confidence level (Kuehl, 2000).

3. Results and discussion

3.1. Effect of treatments on quality indices at day 0 and after storage

Table 2 shows initial mean values and after 14 days of refrigerated storage for all responses obtained for control samples, as well as, mean values for all responses obtained for treated samples under different experimental conditions after 14 days of storage.

At day 0, preservation treatments had no significant initial effect on MES, EB and YM counts, i.e. neither of the combinations evaluated through the experimental design was able to reduce initial counts of strawberry juice (data not shown). This result is in agreement with previous study (Cassani et al., 2016), where vanillin application (1.87 mg/mL) on strawberry juices enriched with fibers did not yield any immediate antimicrobial effect against MES and YM. On the other hand, Tomadoni et al. (2015) evaluated the effect of ultrasound (at 15 and 30 min, 40 kHz, 180 W) on quality parameters of kiwifruit juice during refrigerated storage and they also found no significant differences on MES and YM initial counts between treated and untreated samples. It is noteworthy that the addition of inulin and oligofructose to the juices did not display initial effects on different microbiological indices under study (data not shown). With respect to sensory attributes, the application of vanillin and ultrasound, under different experimental conditions, did not display initial effect on sensory attributes beneath study. In a previous work (Cassani et al., 2016), vanillin applied to strawberry juices enriched with prebiotic fibers affected their typical odor but the concentration used in the later was higher than those used in the present work. It is interesting to point out that the proportion of inulin:oligofructose added to juices did not show significant initial effect on sensory attributes under study.

After 14 days of storage, experiments #5, 7, 9 and 10 showed the highest MES, EB and YM counts and these results were similar to those observed in untreated samples. For these cases, MES and YM counts reached values as high as 10^8 CFU/mL, while EB counts were maintained in 5 logs over storage. Therefore, the combinations of preservation techniques with the formulation of strawberry juice used in these samples were not effective in controlling microbial growth. These samples were enriched with inulin:oligofructose at different proportions and were treated with ultrasound at different times but they have in common that none of them has added vanillin. On the contrary, the lowest MES, EB and YM counts were found in experiments #6, 8, 11 and 12, for which a significant reduction, between 2 and 3 log cycles, was observed in these populations over storage. For these cases, samples were treated with the highest vanillin concentration. Several published works reported that, ultrasound alone is not very effective in killing microorganisms in food since longer treatment times are required (Gómez-López et al., 2010). In a previous work, the efficacy of vanillin in reducing native microflora of strawberry juice enriched with different prebiotics was studied and reductions of 4–6 log cycles compared to untreated samples, after two weeks of storage, were registered (Cassani et al., 2016). With respect to sensory quality, samples obtained from experiments #5, 7, 9 and 10 showed the lowest scores for sensory attributes without significant differences respect to control samples. For these cases, a complete loss of typical odor and OVQ accompanied by the appearance of off-odors occurred during storage. Again, these treated samples correspond to those where no vanillin was added. Therefore, the combined treatment under these experimental conditions, besides having no improvement on the microbiological quality, did not enhance the sensory attributes of strawberry juice. All other samples, even those with the highest vanillin concentration, yielded better sensory quality attributes than control at 14 days of storage, with higher OVQ and typical odor and lower detection of off-odor. It is noteworthy that the upper limit for vanillin concentration was selected based on previous results (Cassani et al., 2016) in order to ensure that it does not present a negative impact on these attributes.

Table 2
Responses of non-treated and treated samples under different experimental conditions after 14 days of storage at 5 °C and initial values of all responses of control samples.

	Sample	Response variables					
		Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Day 0	*C1*	5.76	5.32	5.05	4.87	4.15	0.42
	C2	5.67	5.10	4.96	4.85	4.75	0.27
	C3	5.67	5.23	4.99	4.82	4.55	0.17
Day 14	1	5.23	2.15	5.06	2.57	2.13	1.73
	2	5.13	2.00	4.78	2.53	1.53	2.03
	3	4.71	<2.00	4.19	2.80	1.50	1.53
	4	5.12	2.39	4.44	2.83	1.33	1.00
	5	7.83	5.25	7.93	0.3	0	5
	6	<2.70	<2.00	<2.00	2.57	1.73	1.47
	7	7.89	5.28	7.96	0.6	0	5
	8	3.35	<2	2.30	2.63	1.80	1.27
	9	8.04	4.87	8.56	0.5	0	5
	10	7.92	4.93	8.02	0.3	0	5
	11	3.55	2.15	3.30	3.47	2.83	0.73
	12	<2.70	<2.00	<2.00	3.37	2.53	0.97
	13	4.71	2.15	4.71	3.43	2.47	0.73
	14	4.65	2.15	4.70	3.17	2.57	0.53
	15	4.93	2.45	4.76	3.10	2.63	0.70
	C1	7.78	5.13	7.82	0.4	0	5
	C2	8.14	5.26	8.35	0.5	0	5
	C3	7.80	5.32	7.95	0.3	0	5

Y1: mesophilic bacteria counts, Y2: *Enterobacteriaceae* and total coliforms counts, Y3: yeasts and molds counts, Y4: overall visual quality, Y5: typical odor, Y6: off-odors C1: untreated juice sample; C2: juice sample with an inulin:oligofructose proportion of 1:3 without preservation treatment; C3: juice sample with an inulin:oligofructose proportion of 3:1 without preservation treatment.

3.2. Model fitting

Estimated regression coefficients for quadratic models obtained for each response variable are presented in Table 3. Analysis of variance of each model showed that they were all significant ($p < 0.05$, Table 4). Moreover, the predicted values for all responses calculated from the simplified models, presented a high correlation coefficient with experimental results (data not shown). Hence, these models can be used to describe the effects of the selected independent variables (inulin:oligofructose proportion, ultrasound time and vanillin concentration) on microbiological and sensory quality of strawberry juice.

3.2.1. Microbiological responses

Mesophilic aerobic counts were significantly affected by vanillin concentration ($p < 0.05$). In this sense, increasing vanillin concentration significantly reduced MES counts and a linear effect was observed (Fig. 1A). Vanillin concentration also significantly affected the *Enterobacteriaceae* and total coliforms counts of strawberry juice (linear and quadratic effects) resulting in a positive effect improving microbiological quality (Fig. 1B). Besides, a significant reduction in yeasts and molds counts (linear effect) in samples with increasing vanillin concentration was observed. Fibers proportion also affected the yeasts and molds counts of strawberry juice (linear effect) but in much lower degree (Fig. 1C). In conclusion, all

Table 3
Estimated regression coefficients for RSM analysis of response variables.

	Mesophilic bacteria counts				Yeasts and molds				*Enterobacteriaceae* and total coliform			
Term	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]
Intercept	4.76285	0.409831	11.6215	0.000083***	4.72191	0.324616	14.5461	0.000028***	2.25086	0.339488	6.6301	0.001176**
X_1	−0.52403	0.250969	−2.0880	0.091129	−0.63016	0.198786	−3.1700	0.024814*	−0.48217	0.207893	−2.3193	0.068111
X_1^2	0.19144	0.369417	0.5182	0.626412	0.15757	0.292605	0.5385	0.613323	−0.13327	0.306011	−0.4355	0.681342
X_2	0.46966	0.250969	1.8714	0.120200	0.28634	0.198786	1.4405	0.209287	0.28562	0.207893	1.3739	0.227868
X_2^2	0.09187	0.369417	0.2487	0.813493	−0.26353	0.292605	−0.9006	0.409070	−0.48269	0.306011	−1.5773	0.175542
X_3	−3.10268	0.250969	−12.3628	0.000061***	−3.35880	0.198786	−16.8966	0.000013***	−2.27598	0.207893	−10.9478	0.000111***
X_3^2	−0.07734	0.369417	−0.2094	0.842432	0.08901	0.292605	0.3042	0.773232	0.87074	0.306011	2.8454	0.036016*
$X_1 X_2$	0.12798	0.354924	0.3606	0.733149	0.13154	0.281125	0.4679	0.659549	0.63490	0.294006	2.1595	0.083242
$X_1 X_3$	−0.85555	0.354924	−2.4105	0.060825	−0.68884	0.281125	−2.4503	0.057916	−0.55127	0.294006	−1.8750	0.119637
$X_2 X_3$	0.81305	0.354924	2.2908	0.070581	0.56787	0.281125	2.0200	0.099373	−0.01161	0.294006	−0.0395	0.970036

	Overall visual quality				Typical odor				Off odor			
Term	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]	Coded Coefficient	SE coefficient	*t*-value	*p*-value[a]
Intercept	3.23333	0.189981	17.01929	0.000013***	2.555556	0.192626	13,26690	0.000043***	0.65556	0.168417	3.8925	0.011496*
X_1	0.02917	0.116339	0.25070	0.812019	−0.141667	0.117959	−1,20098	0.283543	−0.12500	0.103134	−1.2120	0.279649
X_1^2	−0.08333	0.171246	−0.48663	0.647101	−0.236111	0.173631	−1,35984	0.231984	0.33056	0.151809	2.1774	0.081375
X_2	0.04583	0.116339	0.39396	0.709848	−0.087500	0.117959	−0,74178	0.491570	−0.05417	0.103134	−0.5252	0.621887
X_2^2	−0.46667	0.171246	−2.72512	0.041521*	−0.694444	0.173631	−3,99954	0.010328*	0.58889	0.151809	3.8791	0.011652*
X_3	1.29167	0.116339	11.10263	0.000103***	1.112500	0.117959	9,43123	0.000226***	−1.94583	0.103134	−18.8671	0.000008***
X_3^2	−1.24167	0.171246	−7.25077	0.000779***	−0.977778	0.173631	−5,63135	0.002447**	1.93889	0.151809	12.7719	0.000052***
$X_1 X_2$	0.01667	0.164528	0.10130	0.923249	0.108333	0.166819	0,64940	0.544724	−0.20833	0.145853	−1.4284	0.212552
$X_1 X_3$	0.02500	0.164528	0.15195	0.885167	−0.075000	0.166819	−0,44959	0.671827	0.05833	0.145853	0.3999	0.705711
$X_2 X_3$	−0.05833	0.164528	−0.35455	0.737390	0.016667	0.166819	0,09991	0.924299	−0.05000	0.145853	−0.3428	0.745683

*: significant with $p < 0.05$; **: significant with $p < 0.01$; ***: significant with $p < 0.001$.
[a] Coefficients with p-value lower than 0.05 were retained in the models.

Table 4
Parameters of models performance evaluation.

Parameter	Y_1 (Mesophilic bacteria counts)	Y_2 (*Enterobacteriaceae* and total coliform)	Y_3 (Yeasts and molds)	Y_4 (Overall visual quality)	Y_5 (Typical Odor)	Y_6 (Off odor)
F-value	19.14	34.36	16.32	20.11	15.17	59.18
p-value	0.0023	0.0006	0.0034	0.0021	0.0040	0.0002
R^2	0.972	0.984	0.967	0.973	0.965	0.991
adjusted R^2	0.921	0.955	0.908	0.925	0.901	0.974

microbiological indices studied were strongly affected by the application of the antimicrobial compound. The inhibitory activity of vanillin resides primarily in its ability to detrimentally affect the integrity of the cytoplasmic membrane, with the resultant loss of ion gradient, pH homeostasis and inhibition of respiratory activity (Rupasinghe, Boulter-Bitzer, Ahn, & Odumeru, 2006). Several works have reported the preservative effect of vanillin (Ferrante et al., 2007; Fitzgerald, Stratford, & Narbad, 2003). Fitzgerald et al. (2003) demonstrated that concentrations of 3.04 and 1.52 mg/mL of vanillin were required to achieve the complete inhibition of both *Saccharomyces cerevisiae* and *Candida parapsilosis* in the apple juice and peach-flavored soft drink over the 8-week storage period at 25 °C. Rupasinghe et al. (2006) also studied the antimicrobial effect of vanillin (*in vitro*) against four pathogenic and spoilage microorganisms that could be associated with contaminated fresh-cut produce. They found that all microorganisms tested were inhibited by vanillin at concentrations between 0.91 and 2.73 mg/mL. Besides, the authors demonstrated that the incorporation of vanillin (*in vivo*) at 1.2 mg/mL into dipping treatments inhibited microbial growth on fresh cut apples during 19 days of storage at 4 °C compared to control.

3.2.2. Sensory responses

Overall visual quality was significantly affected by ultrasound time (quadratic effect) and vanillin concentration (linear and quadratic effects). The quadratic terms of these combined technologies indicate curvatures and nonlinear relationship between the independent variables and OVQ (Fig. 2A). In this sense, OVQ increased with increasing vanillin concentration and ultrasound time up to a certain extent, which were the optimum conditions for obtaining maximum OVQ score. Beyond these values, a decrease in OVQ with vanillin concentration and ultrasound time was observed whatever fibers proportion was assayed. Typical odor was also affected by ultrasound time (quadratic effect) and vanillin concentration (linear and quadratic effects). Fig. 2B shows a strong curvature of the surface due to high significance of quadratic terms. Ultrasound time (up to 15 min) increased typical odor of juice samples, with no further increase using ultrasound time from 15 to 30 min. The same behavior was observed for vanillin concentration. Off-odor was significantly affected by ultrasound time (quadratic effect) and vanillin concentration (linear and quadratic effects, Fig. 2C). The model obtained for this sensory response was opposite to the typical odor model. In this sense, off-odor value significantly decreased with vanillin concentration up to a certain extent

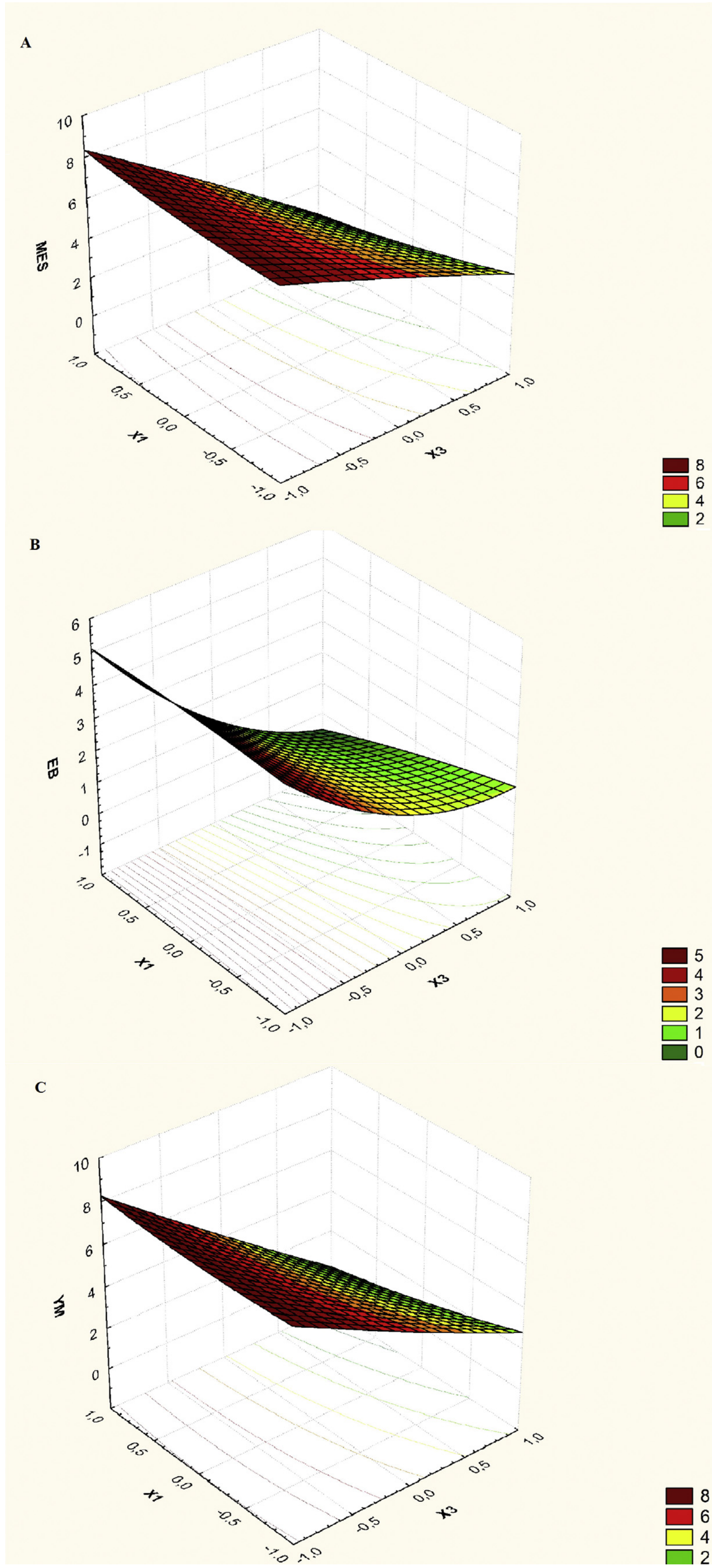

Fig. 1. Response surface plot for the combined effect of fiber proportion and vanillin concentration on: (A) total aerobic mesophilic bacteria counts; (B) *Enterobacteriaceae* and total coliforms counts; (C) yeasts and molds counts of strawberry juices for an ultrasound time at middle level.

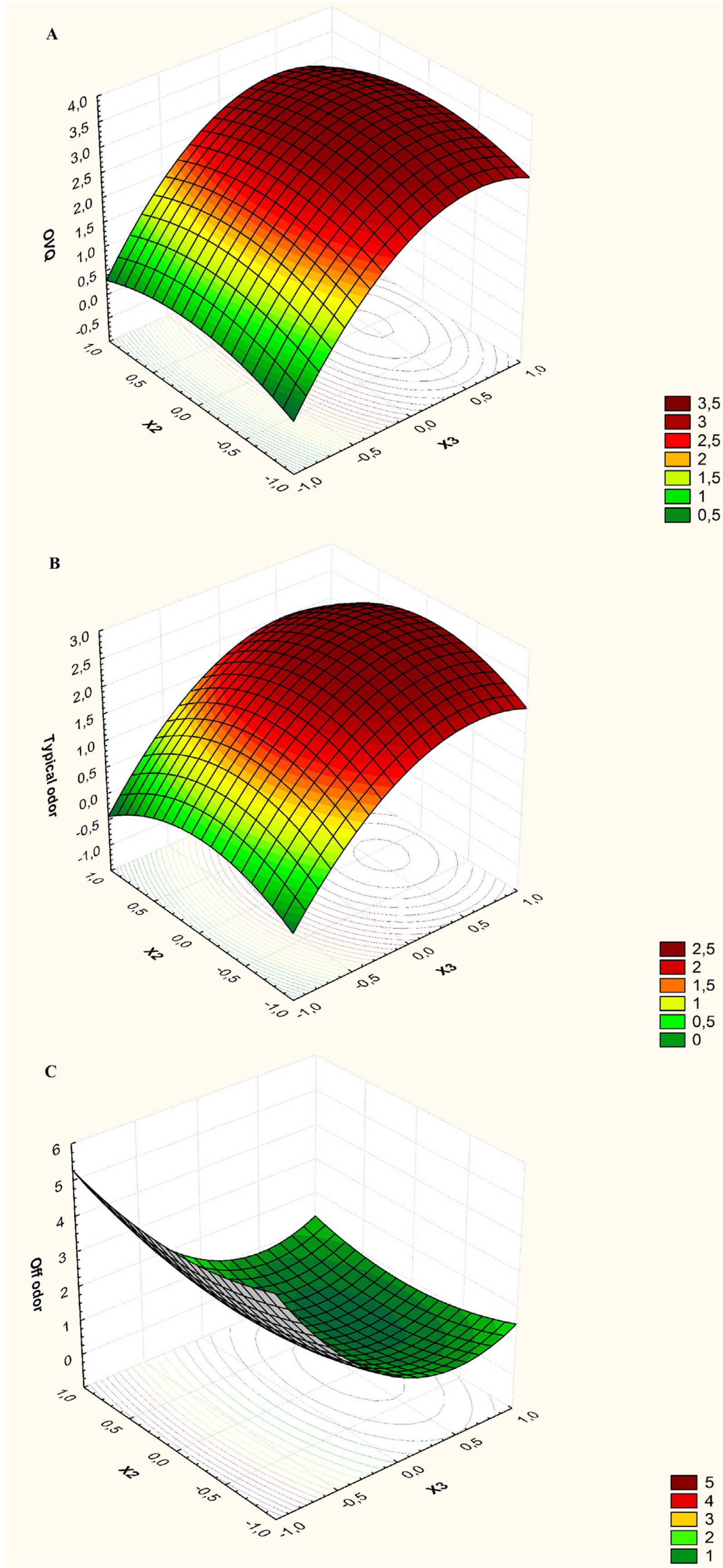

Fig. 2. Response surface plot for the combined effect of ultrasound time and vanillin concentration on: (A) overall visual quality; (B) typical odor; (C) off-odor of strawberry juices for an inulin:oligofructose proportion at middle level.

Table 5
Independent optimization of X1, X2 and X3 for each response and their predicted values carried out under these conditions.

	X1	X2	X3	MES	EB	YM	OVQ	Typical odor	Off-odor
MES	1	−0.5	1	**−0.29**	−0.90	−0.27	3.14	2.05	1.22
EB	1	0	1	0.39	**−0.32**	0.29	3.25	2.24	0.91
YM	1	−0.5	1	−0.29	−0.90	**−0.27**	3.14	2.05	1.22
OVQ	1	0	0.5	2.43	0.44	2.25	**3.53**	2.45	0.40
Typical odor	0	0	0.5	3.19	1.33	3.06	3.57	**2.87**	0.17
Off-odor	1	0.5	0.5	2.96	0.78	2.53	3.43	2.29	**0.41**

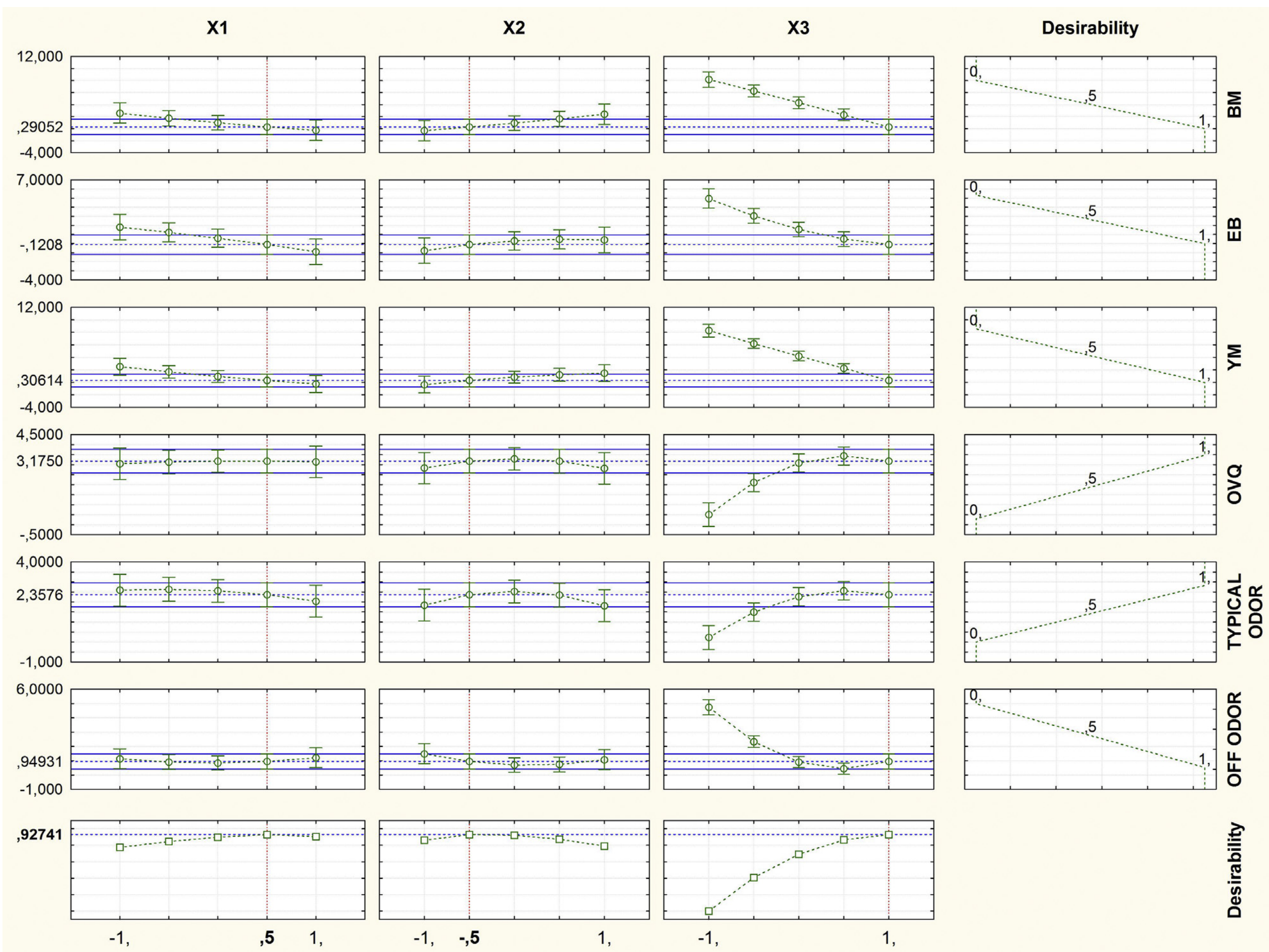

Fig. 3. Profiles for predicted values and Desirability function.

(0.625 mg/mL). Then, off-odor value remained constant for a certain range and finally increased. The positive quadratic coefficient of ultrasound time indicated that off-odor value significantly increased with the increase in this parameter. In conclusion, both preservation techniques positively affected the sensory attributes studied up to a certain extent. Based on our previous works (Cassani et al., 2016; Tomadoni, Viacava, Cassani, Moreira, & Ponce, 2016), vanillin should be used in the lowest concentration possible in order not to modify significantly the organoleptic properties of strawberry juice. In agreement with our finding, Rupasinghe et al. (2006) also indicated that vanillin concentration beyond 1.82 mg/mL could produce unacceptable flavor and aroma for fresh-cut apples. The proportion of inulin:oligofructose added to juices did not show significant interactions with the preservation techniques since sensory attributes under studied, remained unchanged. Therefore, the addition of inulin and oligofructose to the product formulation had no negative implication on sensory quality. This result is in accordance with a previous works, where it was observed that strawberry juice supplemented with prebiotics maintained OVQ and typical odor of the juices (Cassani et al., 2016).

3.3. *Independent and simultaneous optimization of microbiological and sensory responses*

Ridge analysis was conducted to determine the maximum or minimum responses of studied indices depending on the processing variables. Table 5 shows the optimal conditions of X1, X2 and X3 found in the independent optimization for each response and their predicted values, obtained from the models, carried out under these conditions. The microbiological responses showed a similar trend. It is clear from the results that the microorganism inactivation was mostly due to vanillin application since high bioactive concentration (1.25 mg/mL) was required to reduce native microflora. With respect to sensory attributes, middle time of ultrasound and high vanillin concentration allowed obtaining maximum scores for OVQ and typical odor and minimum score for off-odor. It can be observed from Table 5 that when optimizing only YM or MES, low values were found for these variables but the values obtained under these conditions for off-odor was not as good as when optimizing only off-odor. In the same way, when optimizing only OVQ or typical odor, a high value was found for these variables but the values obtained under these conditions for MES or YM were high. These results evidence the necessity of applying simultaneous optimizations.

Fig. 3 shows the results from the simultaneous optimization, using the Desirability function. The optimum formulation and treatment conditions of strawberry juice processing resulted in X1 = 0.5 (inulin:oligofructose proportion of 5:3), X2 = −0.5 (7.5 min of ultrasound), and X3 = 1 (1.25 mg/mL of vanillin), with a desirability value of 0.93. Under these conditions, the investigated responses were theoretically calculated as MES = 1.66 log CFU/mL, EB = 0.84 log CFU/mL; YM = 1.04 log CFU/mL; OVQ = 3.16; typical odor = 2.51; off-odor = 0.79. In this regards, the simultaneous optimization achieves a compromise finding good values for all variables that are being optimized.

3.4. Validation of the simultaneous optimization model

In order to test the reliability of the models in predicting optimal responses, validation experiments were carried out at the optimal levels. The results indicated that the mean experimental values after two weeks of storage were 3.61 for overall quality, 3.36 for typical odor, 1.21 for off-odor, 2.70, 2.00 and 2.39 log CFU/mL for mesophilic, *Enterobacteriaceae* and total coliforms and yeast and molds counts, respectively, at the optimum conditions of inulin:oligofructose proportion, vanillin concentration and ultrasound time obtained after simultaneous validation. It is worth noting that predicted values for microbiological counts were lower than those obtained in the validation experiment. In the same way, OVQ and typical odor scores obtained in the validation experiment were slightly higher than predicted values. These results could be associated to the fact that the batch of strawberries used for validation was harvested in different season, thus environmental factors could affect the initial microbial counts and sensory attributes. A complex interaction between temperature, rain, day duration, background radiation and humidity determines yields and quality of strawberry cultivars in a given region (Pineli, Moretti, Rodrigues, Ferreira, & Chiarello, 2012). In spite of this behavior, it is noteworthy that microbial and sensory indices of samples treated at optimal conditions were all within the acceptability range and were significantly improved respect to control samples. Thus, treated sample at optimal conditions enhanced microbial and sensory attributes of strawberry juice.

4. Conclusion

The inclusion of prebiotic fibers and the application of vanillin and ultrasound treatment were studied for enhancing microbiological and sensory properties of fiber-enriched strawberry juice. Response surface methodology approach was successfully used to study and model the influence of factors (vanillin concentration, ultrasound time and fibers proportion) on microbiological and sensory properties of strawberry juice and simultaneously optimize these factors to obtain an enriched strawberry juice with enhanced microbiological and sensory quality. Microbiological indices were significantly affected by vanillin application whatever ultrasound time and fibers proportion was assayed. Both, bioactive concentration and ultrasound time were found to be critical factors influencing changes in all sensory attributed studied. The inclusion of different prebiotics had no negative implication regarding the quality of fresh juice and no interaction with the preservation techniques was observed. Simultaneous optimization allowed finding a compromise solution among the responses proposing as optimal operating conditions inulin:oligofructose proportion of 5:3, 7.5 min of ultrasound time and 1.25 mg/mL of vanillin. The validation experiments demonstrated that experimentally determined values were in agreement with predicted ones and that juice treated under optimal conditions resulted with an enhanced microbial and sensory attributes.

Conflict of interest

The authors declare that there is no conflict of interest.

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