USE OF ISOMALTULOSE TO FORMULATE HEALTHY SPREADABLE STRAWBERRY PRODUCTS. APPLICATION OF RESPONSE SURFACE METHODOLOGY.

RSM to formulate healthy isomaltulose spreadable strawberry products.

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Abstract

Response surface methodology (RSM) was applied to optimize the formulation of a 50 Brix spreadable strawberry product with healthier sugars and a high percentage of fruit. A central composite design was applied to analyse the influence of four independent variables on the quality parameters. Each of the variables was analysed at five different levels (X1: % of isomaltulose (0, 12.5, 25, 37.5 and 50%), X2: % of pectin (0.5, 1, 1.5, 2 and 2.5%), X3: % of citric acid (0, 0.25, 0.5, 0.75 and 1%) and X4: time of thermal treatment (0, 5, 10, 15 and 20 min). Physicochemical properties, microbiological stability, antioxidant properties (anthocyanin content and antioxidant activity) as well as optical and mechanical properties were considered for the optimization. The influence of storage time on the above parameters was also evaluated. Percentages of pectin and citric acid were the variables that most influenced the measured parameters. In general terms, high levels of these two variables (2% pectin; 0.75% citric acid) resulted in greater antioxidant activity, consistency and adhesiveness values. The optimal formulation to obtain a spreadable strawberry product was fresh strawberry, 50 % fructose, 50 % isomaltulose, 1% citric acid and 1.5% pectin; the ingredients were mixed, and heated to 85ºC, and the product was stable after 90 days stored at room temperature.

Keywords

Isomaltulose, Fructose, Spreadable product, Response surface methodology, Colour and Texture, Antioxidant properties.

Chemical compounds studied in this article

Isomaltulose (PubChem CID: 439559); Sucrose (PubChem CID: 5988); Fructose (PubChem CID: 5984); Pelargonidin-3-glucoside (PubChem CID: 443648); Citric acid (PubChem CID: 311); Potassium sorbate (PubChem CID: 23676745); 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) (PubChem CID: 2735032).

1. Introduction

Nowadays, fruit is receiving ever more attention because of to its high functionality and nutritional value. Among all the available fruits, strawberries are especially interesting due to their content in fibre, minerals, vitamins and other functional compounds such as phenols, many of which have healthy properties. However, strawberries are very seasonal and have a high water content that makes them very perishable (Gillman *et al*., 1995; Cavanah *et al*., 2003; Dhiraj *et al*., 2005).

On the other hand, recent food trends (fast food and ready-to-eat food) have resulted in a decrease in the consumption of fresh fruit, especially among young people. Although there is a wide range of processed fruit products, such as juices, milk based beverages or concentrates, it is important to note that many of them have low fruit content, which in many cases is replaced with a large amount of additives (colorants, flavour enhancers, bulk agents…). Therefore, there is an increased interest for the food industry in developing new processed fruit products with sensory characteristics not very different from those of fresh fruit. For example strawberry spreads, whose characteristics are closer to fresh fruit than other processed products. The main difference between a spreadable fruit product and a jam is that in the former, the step of cooking to reach a final soluble solid content is avoided, as it provokes the greatest changes from a nutritional, sensorial and functional point of view. Moreover, a jam must have at least 45 Brix (B.O.E. 04/07/07; RD: 863/2003), whereas there is no restriction related to sugar content for spreadable fruit products. On the other hand, since sugar consumption is related to health problems such as obesity, one of the goals of the food industry is the replacement of traditional sugars with natural healthier sugars, which have low glycaemic indexes among other functional properties (Sloan, 2005; Peinado *et al*., 2008, 2013). Isomaltulose is a disaccharide that is commercially manufactured enzymatically from sucrose via bacterial fermentation. It is especially indicated for children and senior citizens as it does not produce caries, and moreover is slowly released in the blood (Matsuyama *et al*., 1997; Schiweck, *et al*., 2000; Lina *et al*., 2002). Therefore, isomaltulose could be a healthy replacer of sucrose in the development of fruit products. Since consumers base their choices on the external aspects of the products as well as on their nutritional and functional value, the best formulation of a spreadable strawberry product would be one which provides acceptable colour and texture, but also a large amount of antioxidants. Regarding colour, a brilliant red colour is associated with “freshness” and “healthiness” as opposed to dark red, which may lead to rejection of the product (Cordenunsi *et al*., 2003). Colour stability of this kind of product is affected by temperature, pH, oxygen, sugar content, ascorbic acid and metals (Withy *et al*., 1993). The major pigments in strawberries are pelargonidin-3-glucoside and cyanidin-3-glucoside, which have been reported to have a great contribution to the total antioxidant activity of these products (Wang & Jiao, 2000; Wang & Lin, 2000). The degradation of these pigments may result in discolouration of the product. During processing they can be hydrolysed, and degraded to anthocyanidin and sugar. The anthocyanidins are unstable when exposed to light and are more easily oxidized than the anthocyanin, and consequently more susceptible to browning reactions (Herrmann, 1972). The total amount of these pigments is important for the stability of the colour of the product as well as for their contribution to the total antioxidant activity (Wang & Jiao, 2000; Wang & Lin, 2000). Anthocyanin may react with ascorbic acid, resulting in degradation of both components (Francis, 1985). Finally, regarding texture, this kind of product usually requires the addition of pectin to achieve an adequate gel consistency, so the amount of pectin depends on the amount of sugars and acids as well as the kind of fruit (Rauch, 1987; Gabriele *et al*., 2001; Renard *et al*., 2006).

The aim of this work was to analyse the influence of four independent variables on the quality parameters of 50 Brix spreadable strawberry products after 24h and 90 days of storage. Each of the variables was analysed at five different levels (X_1: % of isomaltulose (0, 12.5, 25, 37.5 and 50%), X_2: % of pectin (0.5, 1, 1.5, 2 and 2.5%), X_3: % of citric acid (0, 0.25, 0.5, 0.75 and 1%) and X_4: time of thermal treatment (0, 5, 10, 15 and 20 min). Physicochemical properties, microbiological stability, antioxidant properties (anthocyanin content and antioxidant activity) as well as optical and mechanical properties were considered for the optimization. Response surface methodology (RSM) was applied to optimize the formulation.

2. Material and Methods

2.1. Raw material

15 Batches of Strawberries (*Fragaria vesca*, Camarosa), were acquired in a local supermarket between February and June 2010 from a supplier who has controlled production areas in various parts of Spain. Each batch of strawberries was sorted in order to eliminate damaged fruit and group the samples in terms of colour, shape and ripeness. Then they were cut and washed in a comercial chlorinated water solution (1% NaHClO), following the indications for the dilutions in order to eliminate possible field residues and microorganisms

2.2 Methodology

2.2.1. Formulation of the spreadable products

According to the response-surface methodology (RSM), a statistical central composite design 2^4 + start (Gómez & Gómez, 1984; Kaur *et al*.,, 2008, Peinado *et al*., 2013) was applied to analyse the influence of four independent variables X_1 (% isomaltulose), X_2 (%pectin), X_3 (%citric acid) and X_4 (time of heat treatment) (Table I) on the quality parameters of the spreadable products. The objective quality parameters were physicochemical properties, microbiologic stability, antioxidant properties (anthocyanin content and antioxidant activity) as well as optical and mechanical properties. This experimental design was applied twice formulating two types of strawberry spread, one group containing sucrose and isomaltulose (with sucrose as the reference sugar, being the control in this group the sample formulated with 100% sucrose) and a second group with fructose and isomaltulose (in which the control was the sample formulated with 100% fructose) (both of them, fructose and isomaltulose, as healthy sugars). The target concentration of the final product was 50 Brix. Therefore, the sweetness of the sugars was not a determinant parameter when the different mixtures where made to formulate the spreadable products. The mixture of isomaltulose with sucrose or fructose was needed in order to reach a final concentration of 50 Brix due to the low solubility of isomaltulose (Kaga & Mizutani, 1985; Schiweck *et al*., 2000). The formulation of the products was the result of the implementation of dry osmotic dehydration (Peinado *et al*., 2012; Rosa *et al*., 2008). In this case, dry osmotic dehydration consists of directly formulating the product by mixing the ingredients in the correct ratio to reach the established concentration.

The ingredients in the spreadable strawberry products were: strawberries, sugars (sucrose or fructose, and isomaltulose), pectin (as a gelling agent), potassium sorbate at a fixed concentration of 500 ppm (as a microbiological preserver) (Karabulut *et al*., 2001; Castelló *et al*., 2006) and citric acid (as a colour preserver). Once the ingredients were mixed, the product was heated just until it reached 85ºC. This temperature was necessary to make hot canning effective, as well as to allow the pectin to dissolve and then gel, but it wasn’t aimed as thermal treatment. Therefore, the gel structure would not break up during storage. Then, the product was placed in glass jars and some of the samples, depending on the statistical design, were heated for 5, 10, 15 or 20 minutes in a bath of boiling water. Finally, they were stored at room temperature, until the correspondent analyses were performed after 24 hours of processing and 90 days of storage.

2.3. Analytical determinations

All measurements were carried out in triplicate.

2.3.1. Physicochemical analyses

Moisture content was determined gravimetrically by drying to constant weight in a vacuum oven at 60ºC (method 20.103 AOAC, 1980). Soluble solids content (Brix) was measured, in previously homogenized samples, with a refractometer at 20ºC (ATAGO 3 T), (dilution at a ratio of 4 g water for each gram of sample was necessary). Water activity (a_w) was determined with a dew point hygrometer (FA-st lab, GBX). pH was determined with a pH-meter (SevenEasy, Mettler Toledo).

2.3.2. Microbiological stability

Shelf-life, from the microbiological point of view, was determined by a modified microbiological analysis method for food and water (Pascual & Calderón, 2000). Mesophilic aerobic populations were analysed in Plate Count Agar by incubating samples for 72 h at 31ºC. Yeast and moulds were determined in Sabouraud chloramphenicol agar plates for 5 days at 31ºC. Sample dilutions were prepared, and after the incubation time, Petri dishes with a number of colonies between 30 and 300 for total counts, and between 0 and 30 for moulds and yeast, were considered.

2.3.3. Texture

Objective determinations of strength and consistency were measured with the back-extrusion test using a texturometer TA/XT/PLUS Texture Analyser and the accessory Back extrusion cell with 35mm ring (García-Martínez *et al*., 2002b; Sesmero *et al*., 2007). The back-extrusion test consisted of making a circular base embolus 35mm in diameter through the sample contained in a cylindrical glass vessel, at a constant speed of 1mm·s^{-1}. For all the assays the embolus covered the same distance to the bottom of the vessel and the amount of sample was the same. This assay provides a typical curve with two characteristic areas. The positive area of the curve is taken as a measurement of consistency, while the negative area corresponds to the adhesiveness but also to the consistency/viscosity of the sample.

2.3.4. Colour

Instrumental measurements of colour were conducted at room temperature in a Minolta spectrophotometer (model CM-3600d), placing the strawberry spread in a 20mm thick, transparent plastic cell and using a black plate as the background to standardize the measurements. Visible absorption spectra were recorded between 380 and 770nm by reflectance to obtain tristimulus values of CIEL*a*b*, using illuminant D_{65} and standard observer (10° visual field) as references.

2.3.5. Anthocyanin content

The anthocyanin content was determined following a modification of the proposed method by Alarcao-E-Silva *et al*., (2001). The sample (1g) was blended in 1% HCl (6 mL) in methanol (80%) and shaken to homogenate it and assure a pH inferior to 2. Then samples were stored at 4ºC for 12h to allow correct separation of the supernatant, which was then filtered through Whatman No. 3 filter paper. Absorbance at 520 nm was measured on the supernatant using 1cm cuvettes and a spectrophotometer (V-630 Jasco). The anthocyanin concentration was correlated with the absorbance of the extract by means of the Lambert Beer law (equation 1). The anthocyanin content was expressed as mg of pelargonidin-(Pgd)-3-glucoside in 100g of raw strawberry or spreadable product, on the basis of the molar extinction coefficient (E_{molar}= 36000M^{-1}cm^{-1}) (Civello *et al*., 1997; Vicente *et al*., 2002) and taking the molecular weight (433g/mol) reported by Skrede, Wrolstad & Enersen, (1992) for pelargonidin-(Pgd)-3-glucoside as it is the major anthocyanin in this fruit (Torreggiani *et al*., 1998) (equation 1):

$$A = a \cdot b \cdot c \qquad \textit{Eq. (1)}$$

Where, A is the absorbance, "c" the concentration (mol/L), "b" thickness of the cuvette (1cm) and "a" the Molar extinction coefficient (E_{molar}).

2.3.6. Antioxidant activity

Antioxidant activity was determined by means of the DPPH method (Castelló *et al*., 2011), which is based on the capacity of the antioxidants to match free radicals. DPPH (2,2-di-phenyl-1-picryhyldrazyl) is a free radical which can react directly with antioxidants and be blocked by them (Antolovich *et al*., 2002; Koleva *et al*.,2002). The reduction of DPPH-H is controlled by the decrease in absorbance of a characteristic wavelength at a determined time during the reaction. In the radical form (DPPH•), it absorbs at 515 nm, but when it is reduced by an antioxidant (-H) or radical species (R•), absorption disappears.

Three grams of sample diluted in 6mL of methanol (80%) in a relation 1:2 (w/v) were centrifuged for 5min at 2200 xG. A solution of 0.024g/L DPPH was prepared. The absorbance of 3.9 mL of the DPPH solution was read at 515nm in a spectrophotometer (V-630 Jasco). Then, 100 μL of supernatant of the spreadable sample diluted in methanol was added to the DPPH solution and absorbance was read again after 60min. The antioxidant capacity results were expressed as inhibition of DPPH (%) (equation 2):

$$\text{Inhibition DPPH}(\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] x\, 100 \qquad \textit{Eq. (2)}$$

where $A_{control}$ = DPPH solution absorbance at 515nm before adding sample, and A_{sample} = DPPH solution absorbance at 515nm after 60 min of adding the sample.

2.3.7. Statistical analysis

Statgraphics Centurion was used to perform the statistical analyses. As has already been mentioned, a central composite design was applied to analyse the influence of four independent variables X_1 (% isomaltulose), X_2 (% pectin), X_3 (% citric acid) and X_4 (time of thermal treatment) at five levels, on the quality parameters of the spreadable products. Statistical analyses of variance (ANOVA) with a confidence level of 95% (p-value $\leq$ 0.05) were also carried out to estimate the significant differences between the raw strawberry's batches used.

Response surface analysis (RSM) was employed to determine the regression coefficients and statistical significance of the model terms as well as to fit the regression models to the experimental data, aiming at an overall optimal region for the response variables studied. After removing the non-significant coefficients from the initial model, the three-dimensional surface plots were used to explain the effects of the independent variables on the response variables. Graphical and numerical optimizations were performed to obtain the optimum conditions and predicted values for the response variables based on the response optimizer.

3. Results and Discussion

3.1. Characterization of Raw material

In order to evaluate the natural variability of the raw material in terms of physicochemical parameters, the different batches were analysed for water activity (a_w), moisture content (x^w), soluble solids content (x^{ss}) and pH, antioxidant activity and anthocyanin content. Regarding physicochemical parameters, the results from the

ANOVAs performed revealed no significant differences between batches in terms of water activity (0.988 ± 0.003), moisture and soluble solids content (x^w: 0.925 ± 0.012; x^{ss}: 0.068 ± 0.006) and pH value (3.45 ± 0.08). Concerning antioxidant activity and anthocyanin content, statistical differences existed between batches. DPPH values varied from 10 ± 2 to 34 ± 2 (% inhibition of DPPH) and anthocyanin content from 10 ± 1 to 48 ± 3 (mg pelargonidin-3-glucosyde/100 g of raw strawberry)**.** These values agree with those found by other authors such as Wang & Lin (2000), Wang & Jiao (2000), Wang & Xu, (2007), Zheng *et al*., (2007), Aaby *et al*. (2007). In these studies, the authors highlighted the highly antioxidant activity of strawberry compounds against peroxyl radicals, hydrogen peroxides and hydroxyl radicals. The antioxidant activity was also found to be strongly affected by crop factors such as growing temperature, CO_2 concentration or the crop system.

3.2. Final composition, pH and water activity of the spreadable strawberry products

After processing, all strawberry spreads showed a very similar composition in terms of soluble solids (x^{ss}) and moisture (x^w) content as they were formulated in order to reach a final concentration of 50 Brix (data not shown). Products formulated with the mixture fructose-isomaltulose exhibited lower values of a_w than those formulated with sucrose-isomaltulose with a same content of isomaltulose. This fact could be attributed to the lower molar mass (180.16 g/mol) and higher solubility of fructose compared with the isomaltulose and sucrose, which have the same molar mass (342.29 g/mol). The higher solubility of fructose leads to a higher reduction of a_w. The pH ranged between 3.03 ± 0.42, for the spreads that had the lowest percentage of citric acid, and 2.80 ± 0.03 for the spreads with the highest percentage of citric acid. These differences could have a direct influence on the stability and consumer acceptance of the product.

3.3. Microbiological stability of the spreadable strawberry products

Microbial counts of mesophilic aerobics, yeasts and moulds were not found in any of the spreadable products during storage ($1x10^4$ ufc/g and $1x10^2$ ufc/g for mesophilic aerobics (31 ± 1ºC) and moulds). This microbiological stability could be attributed to various factors. Firstly, the high soluble solids content leads to a reduction of a_w of the spreadable product compared to fresh strawberries. Secondly, the use of food preservatives such as potassium sorbate and the incorporation of citric acid prevent microorganisms from growing. Thirdly, the thermal treatment (for 5, 10, 15 or 20 min at 100ºC) applied to most of the spreadable products also prevent the growth of microorganisms. However, it is important to point out that no colonies were found in those products in which no thermal treatment was applied (S-5 and F-5). These results make clear that the obtained spreadable strawberry products are microbiologically stable during the analysed period of time.

3.4. Optical and mechanical properties of the spreadable strawberry products

3.4.1. Optical properties

Figure 1 shows the position of the colour parameters of the spread products formulated with 37.5% of isomaltulose (high level of replacement) and the highest and the lowest level of the other parameters (0.25 and 0.75% citric acid (CA); 1 and 2% pectin (P), and 5 and 15 minutes of thermal treatment (min)), after 24h and 90 days of storage, in the colorimetric planes L*- a* and b*-a*. As no significant influence of the percentage of isomaltulose on L*, a* and b* was found, the authors did not consider representation of the rest of products in terms of colorimetric parameters to be necessary.

In general, storage induced a reduction of L*, a* and b* parameters when compared with fresh strawberries, independently of the process variables. Therefore, lower Chroma ($C^*= (a^{*2}+b^{*2})^{1/2}$) and a slight decrease in hue ($h^*= \text{arctg} (b^*/a^*)$) were observed too. The statistical results highlighted that the percentages of citric acid and pectin had a significant effect on the coordinates a* and b* of the spreadable products formulated with the blend sucrose-isomaltulose (p-value < 0.05). The higher the percentages of these variables, the higher the value of these coordinates for the whole of the period studied. Nevertheless, though these two variables had a significant effect on a* and b* values of the spreadable products formulated with the blend fructose-isomaltulose at the beginning of the storage period (p-value < 0.05), this effect disappeared during storage, leading to products with a dark colour, though with a major homogeneity between them. This decrease in the colorimetric coordinates during storage might be due to the higher soluble solids content of strawberry spreads compared with fresh strawberries, and/or the degradation of the red anthocyanin pigments, which are the principal phenolic compounds responsible for strawberry colour (Francis, 1985). In fact, García-Viguera *et al*. (1999) suggested that anthocyanins are very sensitive to different factors such as light, oxygen, temperature, pH, sugar content, ascorbic acid or metals. Moreover, Dervisi *et al*., (2001) observed that the percentage of pectin seems to have an influence on the colour of different jams. It can be said that the influence of the different ingredients on the food system does not only depend on their concentration or distribution within the different system phases but also on the different component interactions during the studied period (Rauch, 1987; Dervisi *et al*., 2001; Renard *et al*., 2006) as can be deduced from the results of this work.

The observed changes in colorimetric coordinates cannot be only attributed to anthocyanin degradations as there are other compounds which also suffer browning reactions (Abers & Wrolstad, 1979); nevertheless, a correlation between anthocyanin content and the colorimetric coordinates was found as other authors had previously proposed (Wicklund *et al*., 2005; Watanabe *et al*., 2011). The best correlation was that obtained for the prediction of the anthocyanin content depending on coordinates a*, b* and L* (a*: $r = 0.81$, $p = 0.000$; b*: $r = 0.66$, $p = 0.000$; L*: $r = 0.19$, $p = 0.0212$) and the percentage of citric acid ($p = 0.003$), (equation 3).

$$\text{Anthocyanin Content} = 4.015-0.217\cdot L^*+2.705\cdot a^*-0.125\cdot b^*-0.063\cdot L^*\cdot a^*-2.048\cdot\%CA \qquad \textit{Eq.(3)}$$

Where, CoAn is the anthocyanin content expressed in mg/100 mg of spreadable product, L*, a* and b* are the colorimetric coordinates and %CA is the percentage of citric acid depending on the experimental design.

3.4.2. Mechanical properties

Once again, no significant influence of the percentage of isomaltulose on consistency and adhesiveness was found after 24h and 90 days of storage. As could be expected, the higher the percentage of pectin, the higher the consistency and adhesiveness values. The presence of acids as well as the increase in temperature strengthened the bounding of the peptide net. Nevertheless, above an optimum acidic value, the elasticity of the gel and its structure may be negatively affected as a result of pectin hydrolysis. Regarding storage, it is important to point out an increase in consistency and adhesiveness during the studied period. Citric acid and pectin, and their interaction, were the only variables with a significant effect on mechanical variables (Figure 2). Nevertheless, it is important to point out that the effect of the different sugars used in the formulation became significant at high levels of pectin. Therefore, spreadable products with greater consistency could be obtained with a sucrose-isomaltulose blend (Figure 2). These results confirmed the role of the food matrix reported above.

3.5. Antioxidant properties

3.5.1. Anthocyanin content

In general, an important decrease in anthocyanin was observed with storage time (Tables 2 & 3). Due to the difference in anthocyanin content between batches of raw material, the initial content of anthocyanin in fresh strawberries was taken into account in order to statistically evaluate the influence of the different variables on the variation of anthocyanin content with storage (Variation of anthocyanin content = $C_{90} - C_{0}$, C_0 being the anthocyanin content in raw material [mg of pelargonidin-(Pgd)-3-glucoside in raw material/100g of raw material] and C_{90} the anthocyanin content in the spreadable product after 90 days of storage [mg of pelargonidin-(Pgd)-3-glucoside in spread product/100g of raw material], respectively). The statistical results showed that the different studied variables had a significant effect on the anthocyanin content of the spreadable products formulated with sucrose-isomaltulose, and especially after 90 days of storage (Figure. 3). However, though the anthocyanin content of the products containing the fructose-isomaltulose blend was influenced by some of the process variables after processing, this effect disappeared at the end of storage. Once again, these results indirectly suggest that the food matrix behaves differently according to the sugar used (sucrose or fructose), and therefore has a varying influence on anthocyanin preservation. In general terms, the percentages of citric acid and pectin were the variables that most influenced anthocyanin preservation. The higher levels of these variables lead to the lowest degradation. These results coincide with those published by other authors such as Patras *et al*., (2009), who observed an increase in anthocyanin content when cranberry purée was heated at 70ºC for two minutes. In the same way, Wesche-Ebeling & Montgomery (1990), Kader *et al*., (1997), and Skrede *et al*., (2000) proved that anthocyanin degradation during the processing of purees increased due to the indirect oxidation of phenolic quinones. The reduction of the anthocyanin degradation associated with the percentage of pectin could be explained as a consequence of the reactions taking place between the pectin, the citric acid and the anthocyanin (*et al*., 2001). In fact, the higher the percentage of pectin, the lower the molecular mobility and the degradation reactions.

3.5.2. Antioxidant activity

The reactions which occur between antioxidant compounds may be additive or synergistic, which is why the measurement of antioxidant activity could offer a global estimation of contribution of the different compounds to global antioxidant capacity (Liu, 2003 and 2004). The antioxidant activity (% DPPH inhibition) of the different spreadable products was estimated (Tables 2 & 3). Immediately after formulation (24 h of storage), the spreadable product presented similar antioxidant activity to raw strawberries. However, a considerable increase was reported after 90 days of storage for most products.

The statistical results showed no influences of the percentage of isomaltulose and time of thermal treatment (TT) on the antioxidant activity, citric acid and pectin being the variables which most influenced this parameter (Figure 4). In general terms, a high antioxidant capacity was associated with a high level of pectin and citric acid in the product. Nevertheless, the interaction of both parameters revealed that for the highest percentages of pectin, the highest DPPH values were reached with a low percentage of citric acid in the product. For spreadable products formulated with low percentages of citric acid, the strength of the tri-dimensional network (or the gel) becomes a key-factor for the retention and protection of the antioxidant components against oxidative agents (light, oxygen, etc). This increase of antioxidant activity during storage could be explained taking into account that it is not only dependent on the anthocyanin content, but also on other compounds which contributed to the antioxidant activity and could increase or be generated during storage. In fact, the generation of new compounds with high antioxidant activity from ellagic acid might result in an increase of antioxidant activity during storage. These results coincide with those reported by other authors who found a decrease in anthocyanin content while global antioxidant activity increased during the storage of strawberry jams and purees at room temperature (Aaby *et al.* 2007, Kalt, 2005; García-Alonso *et al*.,2003; Mullen *et al*., 2002; Gil *et al*., 2000).

3.6. Optimization of the formulation

Once the influence of the different process variables on physicochemical, optical and mechanical properties, microbiological stability, anthocyanin content and antioxidant activity were performed, the following targets were established to carry out the optimization. Firstly, it was decided to maximize the total antioxidant activity as it is not only dependent on anthocyanin content. Regarding colour, the colorimetric coordinates of raw strawberries were selected as the target, because the original red colour of strawberries was required in the final product. Concerning mechanical properties, the selected values for consistency and adhesiveness were those which gave an appropriate spreadability of the gel when compared to similar commercial products such as jams. Finally, it was decided to set up the levels of isomaltulose and time of thermal treatment; therefore, the highest percentage of isomaltulose was selected (as the objective of this work was the maximum replacement of sucrose by healthier sugars); no thermal treatment was selected as there was no risk of microbiological growth. Figure 5 illustrates the set up parameters for the optimization as well as the optimum percentages of pectin and citric acid for the highest possible response. It can be observed that regardless of the sugar used, the required percentages of pectin and citric acid were similar. Thus, to obtain a spreadable strawberry product with the maximum sucrose replacement by isomaltulose (50% isomaltulose and 50% sucrose or fructose), a formulation with 1% citric acid and 1.5% pectin is necessary, without thermal treatment after hot canning.

4. Conclusions.

The levels of pectin and citric acid had the greatest influence on the quality parameters of the spreadable strawberry products. The spreadable products presented similar antioxidant activity to raw strawberries, which increased during the storage period. Concerning colour, storage resulted in a darkening of the products in general. The colorimetric coordinates of the products containing the sucrose-isomaltulose blend seemed to be influenced by the percentages of pectin and citric acid while the colour of the samples containing the fructose-isomaltulose blend, did not seem to be affected by the different variables. Finally, high levels of pectin and citric acid produced products with high values for consistency and adhesiveness, as expected. After optimization of the process, it can be concluded that a spreadable strawberry product formulated with healthy sugars could be obtained by formulation with the fructose-isomaltulose blend for the maximum percentage of isomaltulose (50% isomaltulose and 50% fructose), with 1% citric acid and 1.5% pectin.

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'Conflict of Interest Statement'

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other people who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Table I. Independent variables and levels of the central composite design.

Independent variables	Symbol[c]	Coded variable levels				
		-2	**-1**	**0**	**1**	**2**
Isomaltulose (%)[a]	X_1	0	12.5	25	37.5	50
Pectin (%)[b]	X_2	0.5	1	1.5	2	2.5
Citric acid (%)[b]	X_3	0	0.25	0.5	0.75	1
Heat treatment time (min)	X_4	0	5	10	15	20

a: % of isomaltulose in the total amount of sugar mix (sucrose-isomaltulose or fructose-isomaltulose).
b: in final product.
c: Symbol with which each independent variable is cited in the text.

Table 2. Antioxidant values (anthocyanin's [mg of Pgd-3-g /100g spreadable product] & DPPH [% DPPH Inhibition] and mechanical values (Consistency and Adhesiveness [N.s]). Combination of the different variables performed to obtain 26 products with fructose. (n=3).

Code		X_1	X_2	X_3	X_4	Anthocyanin's (mg of Pgd-3-g /100g)		DPPH (% Inhibition)		Texture (Ns) Consistency		Texture (Ns) Adhesiveness	
	% F[a]					t=0 days	t= 90 days	t=0 days	t= 90 days	t=0 days	t= 90 days	t=0 days	t= 90 days
F-1	62	37.5	0.75	2	15	8.560 (0.080)	1.357 (0.106)	22.700 (0.300)	93.900 (0.400)	11.000 (1.000)	35.000 (1.000)	2.300 (0.200)	4.700 (0.200)
F-2	62.5	37.5	0.75	2	5	7.800 (0.200)	2.090 (0.090)	9.600 (0.400)	92.800 (0.500)	6.600 (0.300)	13.040 (0.070)	1.500 (0.080)	2.240 (0.020)
F-3	75	25	1	1.5	10	8.060 (0.400)	1.100 (0.070)	23.000 (2.000)	94.500 (0.400)	4.260 (0.090)	7.560 (0.060)	0.730 (0.030)	1.250 (0.020)
F-4	62.5	37.5	0.75	1	5	9.300 (0.200)	1.200 (0.200)	22.000 (5.000)	94.000 (0.400)	3.790 (0.080)	4.640 (0.080)	0.590 (0.020)	0.700 (0.020)
F-5	75	25	0.5	1.5	0	9.600 (0.200)	1.500 (0.300)	14.000 (1.000)	97.000 (0.200)	5.800 (0.300)	5.370 (0.120)	1.210 (0.090)	0.993 (0.006)
F-6	62.5	37.5	0.75	1	15	9.200 (0.300)	1.700 (0.200)	18.000 (4.000)	95.000 (1.000)	3.700 (0.060)	4.750 (0.040)	0.618 (0.012)	0.714 (0.013)
F-7	87.5	12.5	0.75	1	15	8.800 (0.200)	1.810 (0.140)	12.000 (0.300)	96.600 (0.200)	3.810 (0.060)	5.050 (0.040)	0.655 (0.009)	0.720 (0.009)
F-8	100	0	0.5	1.5	10	9.000 (1.000)	1.300 (0.200)	17.000 (2.000)	96.700 (0.400)	5.310 (0.020)	4.537 (0.012)	1.115 (0.014)	0.828 (0.013)
F-9	75	25	0.5	0.5	10	10.900 (0.200)	1.730 (0.070)	14.000 (3.000)	97.000 (0.500)	2.850 (0.060)	2.850 (0.060)	0.306 (0.012)	0.234 (0.004)
F-10	87.5	12.5	0.75	1	5	14.900 (0.600)	2.200 (0.120)	6.000 (0.900)	96.400 (0.800)	3.380 (0.003)	3.700 (0.080)	0.516 (0.009)	0.517 (0.005)
F-11	75	25	0	1.5	10	13.500 (0.200)	2.380 (0.080)	16.000 (2.000)	93.200 (0.700)	5.170 (0.060)	4.540 (0.040)	1.081 (0.013)	0.851 (0.012)
F-12	75	25	0.5	1.5	10	11.600 (0.200)	1.980 (0.130)	21.000 (1.000)	92.800 (0.600)	5.120 (0.040)	5.710 (0.080)	1.074 (0.012)	1.140 (0.020)
F-13	87.5	12.5	0.25	1	15	8.400 (0.200)	1.510 (0.090)	13.000 (1.000)	92.700 (0.400)	3.890 (0.020)	3.300 (0.200)	0.684 (0.012)	0.503 (0.003)
F-14	75	25	0.5	1.5	10	11.570 (0.140)	2.160 (0.130)	25.000 (3.000)	92.100 (0.200)	5.320 (0.090)	5.910 (0.060)	1.110 (0.020)	1.183 (0.007)
F-15	62.5	37.5	0.25	1	5	12.000 (1.000)	1.900 (0.200)	17.300 (0.900)	92.800 (0.400)	4.000 (0.200)	3.430 (0.040)	0.700 (0.060)	0.480 (0.020)
F-16	87.5	12.5	0.25	1	5	15.200 (0.500)	2.400 (0.200)	17.000 (4.000)	92.500 (0.700)	3.810 (0.120)	3.550 (0.080)	0.637 (0.008)	0.544 (0.008)
F-17	62.5	37,5	0.25	1	15	8.800 (0.300)	1.000 (0.200)	14.000 (2.000)	93.400 (0.700)	3.430 (0.080)	3.110 (0.040)	0,55 (0.020)	0.422 (0.013)
F-18	87,5	12,5	0.25	2	5	7.900 (0.800)	2.220 (0.130)	22.840 (0.060)	92.100 (0.300)	6.200 (0.200)	5.560 (0.103)	1.405 (0.012)	1.230 (0.020)
F-19	75	25	0.5	2.5	10	13.000 (2.000)	2.330 (0.020)	32.000 (1.000)	92.700 (0.900)	10.720 (0.130)	20.300 (0.600)	2.460 (0.030)	3.570 (0.090)
F-20	87.5	12.5	0.75	2	15	14.300 (0.800)	2.300 (0.200)	30.000 (2.000)	92.700 (0.900)	6.000 (0.200)	9.000 (0.070)	1.362 (0.008)	1.690 (0.060)
F-21	87.5	12.5	0.25	2	15	8.840 (0.800)	1.900 (0.200)	23.700 (0.500)	93.800 (0.800)	7.610 (0.050)	6.700 (0.200)	1.800 (0.020)	1.578 (0.002)
F-22	87.5	12.5	0.75	2	5	13.000 (0.300)	1.880 (0.050)	31.000 (3.000)	92.600 (0.700)	7.600 (0.090)	17.770 (0.080)	1.650 (0.030)	2.947 (0.014)
F-23	75	25	0.5	1.5	20	6.800 (0.300)	1.590 (0.120)	11.700 (0.700)	94.000 (1.000)	4.950 (0.070)	3.970 (0.140)	1.010 (0.020)	0.792 (0.008)
F-24	62.5	37.5	0.25	2	15	7.700 (0.370)	1.800 (0.300)	29.000 (2.000)	93.400 (0.400)	7.100 (0.200)	5.680 (0.090)	1.670 (0.030)	1.350 (0.020)
F-25	62.5	37.5	0.25	2	5	6.300 (0.400)	1.020 (0.140)	14.500 (0.130)	92.800 (0.500)	8.500 (0.600)	6.000 (0.300)	2.000 (0.200)	1.430 (0.020)
F-26	50	50	0.5	1.5	10	4.600 (0.400)	1.100 (0.200)	24.000 (0.200)	93.400 (0.900)	4.500 (0.20)	4.180 (0.090)	0.93 (0.05)	0.814 (0.009)

a: mixture of sugars to reach 50 ºBrix in the spreads: 100% of sugar = fructose % + isomaltulose %.
F: Fructose; I: Isomaltulose.
X_1 (% of isomaltulose), X_2 (% of pectin), X_3 (% of citric acid) and X_4 (time of thermal treatment (min)).
Anthocyanin's: mg of pelargonidin-(Pgd)-3-glucoside in 100g of spreadable product.

Table 3. Antioxidant values (anthocyanin's [mg of Pgd-3-g /100g spreadable product] & DPPH [% DPPH Inhibition] and mechanical values (Consistency and Adhesiveness [N.s]). Combination of the different variables performed to obtain 26 products with sucrose. (n=3).

Code		X_1	X_2	X_3	X_4	**Anthocyanin's** (mg of Pgd-3-g /100g)		**DPPH** (% Inhibition)		**Texture (Ns)** Consistency		Adhesiveness	
	% S[a]					**t=0 days**	**t= 90 days**	**t=0 days**	**t= 90 days**	**t=0 days**	**t= 90 days**	**t=0 day**	**t= 90 days**
S-1	62	37.5	0.75	2	15	9.800 (0.200)	3.060 (0.120)	21.000 (2.000)	93.700 (0.700)	24.000 (4.000)	40.000 (2.000)	4.200 (0.700)	5.420 (0.080)
S-2	62.5	37.5	0.75	2	5	9.070 (0.060)	3.400 (0.200)	13.300 (0.200)	90.000 (3.000)	7.600 (0.200)	20.100 (0.500)	1.330 (0.040)	2.710 (0.040)
S-3	75	25	1	1.5	10	11.300 (0.500)	4.200 (0.300)	19.000 (0.500)	92.800 (0.900)	5.120 (0.040)	13.500 (0.200)	0.865 (0.013)	1.777 (0.014)
S-4	62.5	37.5	0.75	1	5	10.600 (0.740)	3.170 (0.020)	20.000 (1.000)	93.650 (0.120)	3.600 (0.300)	5.340 (0.060)	0.500 (0.080)	0.717 (0.013)
S-5	75	25	0.5	1.5	0	11.070 (0.200)	3.700 (0.400)	14.000 (2.000)	96.800 (0.200)	5.220 (0.120)	8.830 (0.030)	0.980 (0.040)	1.444 (0.013)
S-6	62.5	37.5	0.75	1	15	9.800 (0.400)	2.040 (0.300)	15.000 (0.500)	97.100 (0.300)	3.240 (0.070)	5.500 (0.200)	0.410 (0.020)	0.738 (0.007)
S-7	87.5	12.5	0.75	1	15	10.500 (0.200)	4.100 (0.400)	11.000 (1.000)	95.600 (0.800)	3.630 (0.030)	5.740 (0.120)	0,517 (0,012)	0.813 (0.014)
S-8	100	0	0.5	1.5	10	10.000 (1.000)	4.000 (0.200)	12.000 (1.000)	96.000 (3.000)	5.200 (0.200)	6.280 (0.090)	1.010 (0.050)	1.087 (0.012)
S-9	75	25	0.5	0.5	10	12.500 (0.090)	3.500 (0.300)	13.000 (1.000)	96.600 (0.300)	3.130 (0.030)	2.980 (0.020)	0.353 (0.007)	0.247 (0.002)
S-10	87.5	12.5	0.75	1	5	17.700 (0.600)	5.500 (0.400)	13.000 (2.000)	96.800 (0.600)	3.380 (0.080)	4.340 (0.200)	0.460 (0.020)	0,571 (0,007)
S-11	75	25	0	1.5	10	14.700 (0.300)	5.100 (0.500)	16.000 (1.000)	93.600 (0.700)	6.160 (0.130)	5.620 (0.090)	1.320 (0.050)	1.106 (0.006)
S-12	75	25	0.5	1.5	10	11.500 (0.350)	4.260 (0.080)	15.973 (0.989)	92.700 (0.400)	7.900 (0.300)	18.100 (0.200)	1.470 (0.040)	2.690 (0.070)
S-13	87.5	12.5	0.25	1	15	11.470 (0.103)	4.100 (0.400)	12.000 (1.000)	93.100 (0.600)	3.820 (0.060)	3.730 (0.120)	0.640 (0.007)	0.609 (0.003)
S-14	75	25	0.5	1.5	10	14.040 (0.050)	4.900 (0.300)	16.000 (1.000)	93.100 (0.300)	5.820 (0.200)	9.180 (0.050)	1.140 (0.050)	1.540 (0.030)
S-15	62.5	37.5	0.25	1	5	12.300 (0.300)	3.290 (0.070)	11.900 (0.300)	93.300 (0.800)	4.060 (0.080)	3.720 (0.020)	0.660 (0.020)	0.528 (0.012)
S-16	87.5	12.5	0.25	1	5	13.500 (0.500)	5.500 (0.200)	15.000 (3.000)	92.900 (0.800)	3.810 (0.020)	3.790 (0.090)	0.598 (0.004)	0.600 (0.020)
S-17	62.5	37,5	0.25	1	15	8.000 (1.000)	2.660 (0.012)	10.500 (0.200)	92.800 (0.200)	3.570 (0.070)	3.370 (0.050)	0.571 (0.014)	0.490 (0.020)
S-18	87,5	12,5	0.25	2	5	10.500 (0.300)	2.600 (0.130)	25.000 (2.000)	91.000 (2.000)	9.130 (0.090)	9.270 (0.120)	2.070 (0.050)	1.966 (0.006)
S-19	75	25	0.5	2.5	10	11.400 (0.400)	5.100 (0.400)	29.000 (2.000)	93.820 (0.140)	16.300 (0.300)	37.000 (2.000)	3.140 (0.090)	5.700 (0.200)
S-20	87.5	12.5	0.75	2	15	9.500 (0.600)	5.500 (0.200)	38.500 (0.400)	93.200 (0.300)	7.898 (0.014)	19.300 (0.800)	1.406 (0.013)	2.740 (0.060)
S-21	87.5	12.5	0.25	2	15	8.100 (0.500)	3.960 (0.120)	12.000 (2.000)	93.600 (0.400)	7.700 (0.200)	7.550 (0.090)	1.770 (0.020)	1.626 (0.013)
S-22	87.5	12.5	0.75	2	5	10.200 (0.620)	4.300 (0.300)	14.000 (4.000)	93.000 (0.800)	7.900 (0.300)	17.800 (0.400)	1.460 (0.040)	2.444 (0.112)
S-23	75	25	0.5	1.5	20	10.700 (0.680)	4.600 (0.500)	10.000 (2.000)	93.900 (0.600)	5.500 (0.200)	8.470 (0.070)	1.060 (0.020)	1.380 (0.020)
S-24	62.5	37.5	0.25	2	15	10.500 (0.500)	3.400 (0.500)	14.000 (5.000)	93.100 (0.200)	6.5600 (0.200)	6.900 (0.200)	1.540 (0.030)	1.501 (0.020)
S-25	62.5	37.5	0.25	2	5	7.200 (0.800)	2.800 (0.200)	14.000 (2.000)	93.600 (0.500)	7.404 (0.104)	5.800 (0.120)	1.640 (0.030)	1.227 (0.014)
S-26	50	50	0.5	1.5	10	6.300 (0.200)	3.800 (0.200)	15.000 (1.000)	93.700 (0.800)	4.930 (0.120)	5.180 (0.050)	1.010 (0.050)	0.975 (0.009)

a: mixture of sugars to reach 50 ºBrix in the spreads: 100% of sugar = sucrose % + isomaltulose %.
S: Sucrose; I: Isomaltulose.
X_1 (% of isomaltulose), X_2 (% of pectin), X_3 (% of citric acid) and X_4 (time of thermal treatment (min)).
Anthocyanin's: mg of pelargonidin-(Pgd)-3-glucoside in 100g of spreadable product.

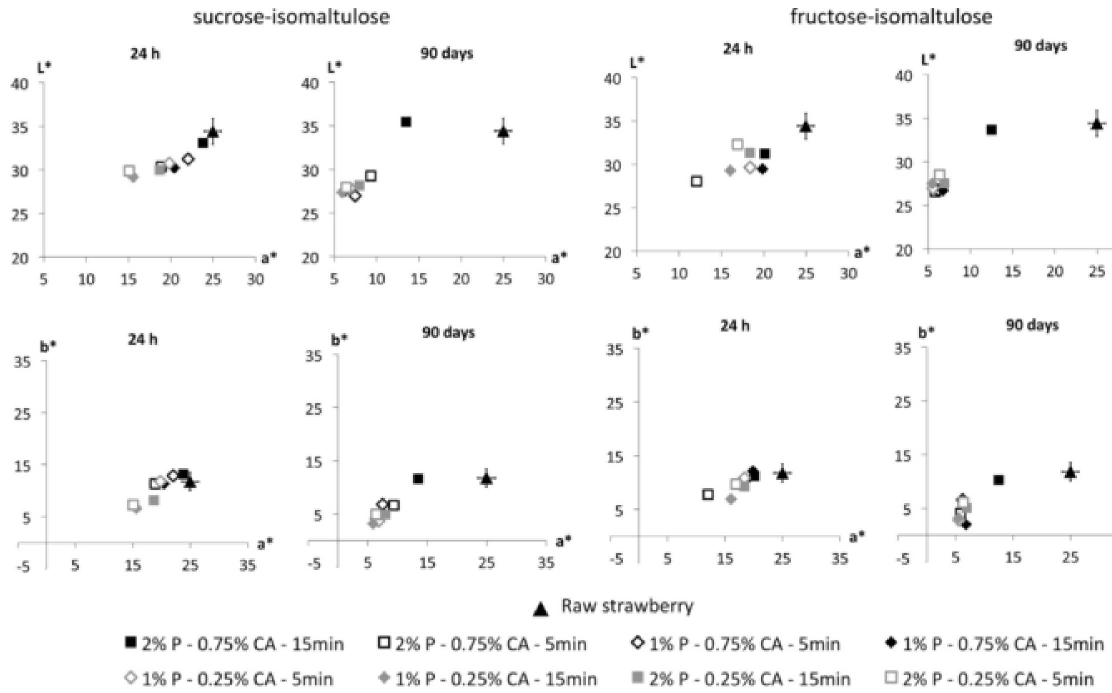
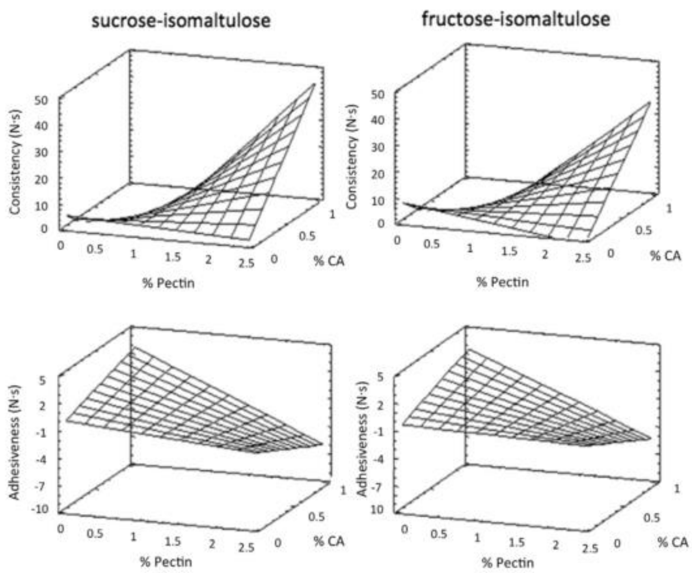


Figure 1. Representation of the colorimetric parameters L*, a* and b* of the raw material and strawberry spreadable products formulated with fructose-isomaltulose (code: F-number) and sucrose-isomaltulose (code: S-number) at 37.5% of isomaltulose in the colorimetric planes L*-a* and b*-a*. The shown values correspond to products formulated with 1 and 2% of pectin (P), 0.25 and 0.75% of citric acid (CA), and 5 and 15 minutes of thermal treatment (min)

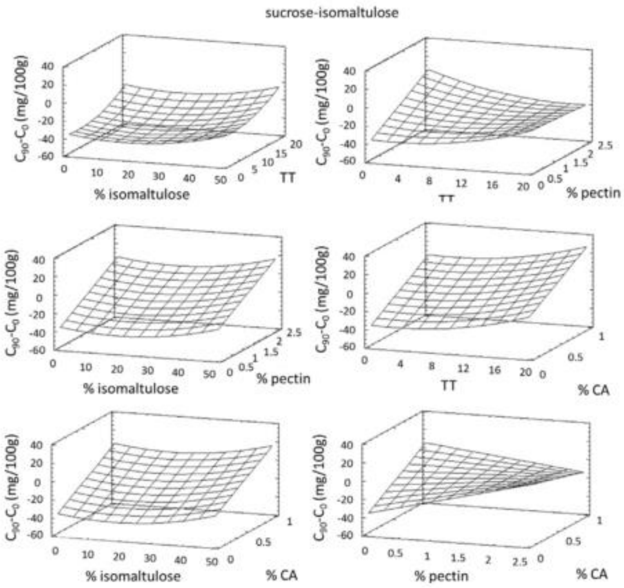


Variables	t=0 days		t=90 days	
	F_{ratio}	P_{value}	F_{ratio}	P_{value}
sucrose-isomaltulose				
Consistency				
% Pectin	21.13	**0.0001*** **	50.56	**0.0000*** **
% Citric	-	-	15.20	**0.0008*** **
%P x %P	-	-	8.59	**0.0080** **
% P x % CA	-	-	10.05	**0.0046** **
Adhesiveness				
% Pectin	180.17	**0.0000*** **	66.59	**0.0000*** **
% Citric			7.35	**0.0131***
%P x %P	5.21	**0.0320***	8.33	**0.0088** **
% P x % CA			5.22	**0.0328***
fructose-isomaltulose				
Consistency				
% Pectin	117.14	**0.0000*** **	14.68	**0.0009*** **
%Citric	-	-	5.68	**0.0262***
% P x % P	6.62	**0.0170***	-	-
% P x % CA	-	-	5.02	**0.0355***
Adhesiveness				
% Pectin	180.17	**0.0000*** **	33.78	**0.0000*** **
%Citric	-	-	5.69	**0.0261***
% P x % P	5.21	**0.0320***	-	-
% P x % CA	-	-	4.58	**0.0437***

Iso: Isomaltulose; TT: Thermal treatment; P: Pectin; CA: Citric

*sig > 0.95; ** sig > 0.99; *** sig > 0.999.

Figure 2. Response-Surface plots for the consistency values (N·s) and adhesiveness (N·s) of the strawberry spreadable products after 90 days of storage depending on the percentage of pectin and citric acid



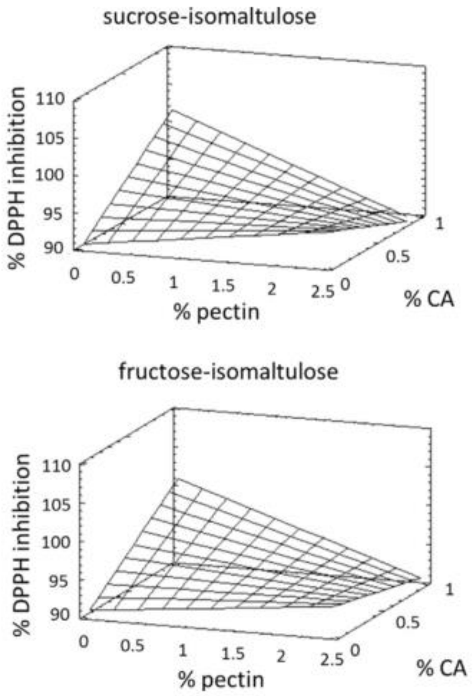
Variables	t=0 days		t=90 days	
	F_{ratio}	P_{value}	F_{ratio}	P_{value}
sucrose-isomaltulose				
C_F	**4.54**	**0.0002*** **	**2.77171**	**0.0106***
% Iso	-	-	4.96	**0.0397***
% Pectin	1.08	0.3105	9.77	**0.0062** **
% Citric	9.10	**0.0066** **	13.61	**0.0018** **
TT	8.84	**0.0073** **	0.36	0.5556
% Iso x % Iso	-	-	9.70	**0.0063** **
TT x TT	-	-	6.81	**0.0183***
% P x % CA	7.68	**0.0114***	6.53	**0.0205** **
TT x % P	-	-	11.30	**0.0037** **

Iso: Isomaltulose; TT: Thermal treatment; P: Pectin; CA: Citric

CF: Anthocyanin's content in the raw batches.

*sig > 0.95; ** sig > 0.99; *** sig > 0.999.

Figure 3. Response-Surface plots for the variation of anthocyanin concentration for the strawberry spreadable products formulated with sucrose-isomaltulose after 90 days of storage.



Variables	t=0 days F_{ratio}	t=0 days P_{value}	t=90 days F_{ratio}	t=90 days P_{value}
sucrose-isomaltulose				
% Iso	-	-	-	-
% Pectin	7.18	**0.0131***	1.14	0.2963
%Citric	-	-	6.33	**0.0197***
TT	-	-	-	-
% P x % CA	-	-	5.34	**0.0307***
fructose-isomaltulose				
% Iso	8.25	**0.0094****	-	-
% Pectin	18.93	**0.0003*****	0.72	0.4041
%Citric	-	-	6.57	**0.0177***
TT	6.03	**0.0234***	-	-
TT x TT	5.55	**0.0287***	-	-
% Iso x % Iso	-	-	-	-
% Iso x % P	8.49	**0.0086****	-	-

Iso: Isomaltulose; TT: Thermal treatment; P: Pectin; CA: Citric

*sig > 0.95; ** sig > 0.99; *** sig > 0.999.

Figure 4. Response-Surface plots for the antioxidant activity (% DPPH inhibition) of the strawberry spreadable products after 90 days of storage depending on the percentage of pectin and citric acid

Response	goal	Optimal values sucrose-isomaltulose	Optimal values fructose-isomaltulose
L* (Luminosity)	35.0	27.96	29.48
a* (red-green colour)	20.0	9.10	9.11
b* (yellow-blue colour)	10.0	7.71	7.20
Antioxidant Activity	Maximize	94.42	94.66
Consistency	15.0	15.35	13.12
Adhesiveness	2.0	2.00	2.00

Antioxidant activity: (% Inhibition DPPH); Consistency and Adhesiveness (N·s)

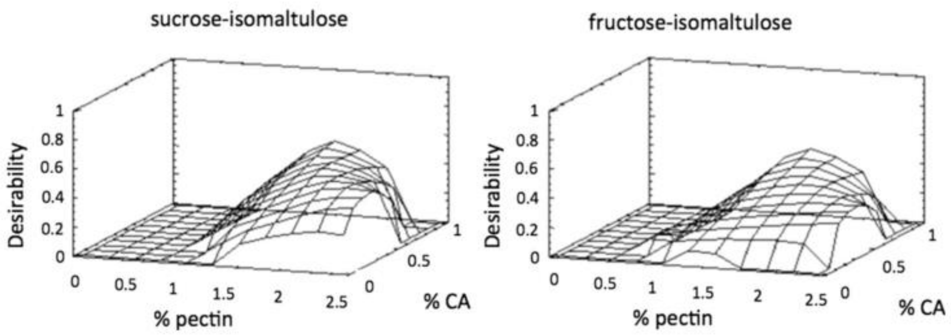


Figure 5. Set up parameters for the optimization and Response-Surface plots obtained after the optimization depending on the percentages of pectin and citric acid.