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Natural food colorant from blackcurrant spray-dried powder obtained by enzymatic treatment: characterization and acceptability.

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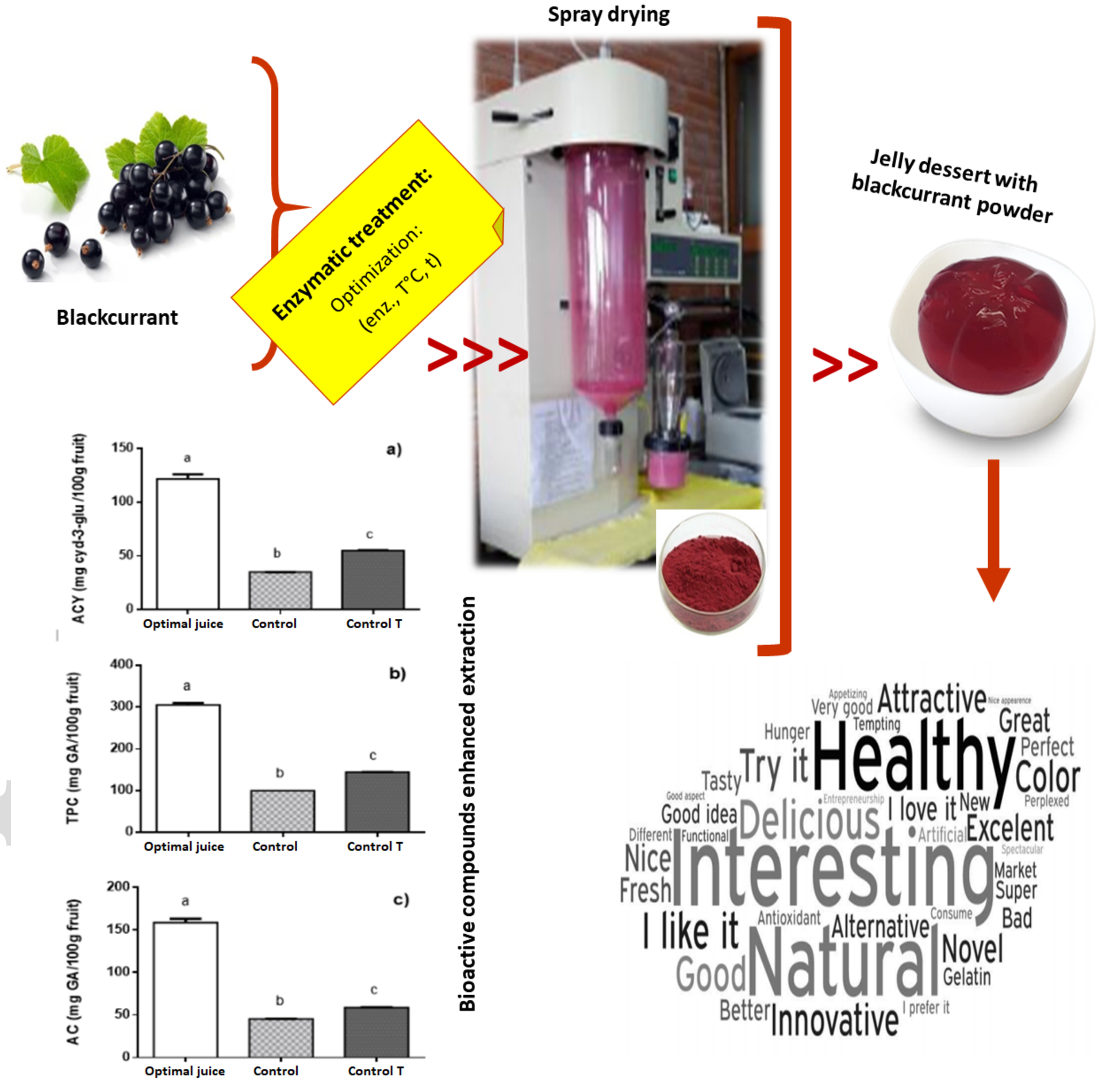
Graphical Abstract



Abstract

Blackcurrant juice extraction was optimized by enzymatic maceration. A Box-Behnken experimental design was used considering the effect of enzyme concentration, temperature and treatment time over the total polyphenolic content (TPC), antioxidant capacity (AC), and juice yield. Under the obtained optimal conditions (200 ppm enzyme, 45°C and 60 min) a 2.4-fold increment for TPC, 2.8-fold for AC and 2.7-fold for total monomeric anthocyanins (ACY) were observed, along with a 15% increase in juice yield. The juice was spray-dried and the powder was tested as an ingredient in a jelly dessert. An attractive color product was obtained, also containing blackcurrant bioactive compounds (0.56±0.03 mg GA/g, 0.20±0.02 mg cyd-3-glu/g and 0.16±0.02 mg of GA/g for TPC, ACY and AC, respectively). This jelly was stable regarding syneresis and antioxidant compounds for 15 days at 4°C. The developed blackcurrant powder could be useful as natural colorant and source of bioactive compounds for several food applications.

Keywords: Blackcurrant; enzymatic extraction; bioactive compounds; spray-drying; food ingredients.

Practical Application

An efficient extraction process of blackcurrant juice by enzymatic treatment of ground fruit was achieved. As a result of the process it was possible to improve the extraction of bioactive compounds and also increasing antioxidant activity. A fruit juice powder was obtained from the enriched juice by spray drying. The blackcurrant powder showed relatively good physical characteristics and potential functional properties. The use of this powder as a natural colorant in a jelly dessert showed promising results. The ingredient not only enriched the product with

bioactive components, but also provided a stable and attractive color. Moreover, the gel properties were improved and the jelly showed very good acceptance in the consumer's perception test. These results prove that this ingredient has good potential to be used as natural colorant and functional additive in food.

1. Introduction

In accordance to the worldwide interest towards healthier foods, the market for food colorants is giving rise to the search for natural alternatives to replace artificial food colors. Berries have been the object of interest due to their high content of bioactive compounds that provide antioxidant activity, and are known to confer many health benefits like anti-inflammatory, immunomodulatory, antimicrobial, improvement of vision, and neuroprotective effects (Gopalan et al., 2012). Among these fruits, blackcurrant is recognized for its high concentration of anthocyanins, water-soluble natural pigments (He and Giusti, 2010). Their colors range from red to violet, and show high stability at low pHs (Bakowska-Barczak, 2005); making them suitable colorants for acidic foods.

The use of pectinolytic enzymes is based on their capacity to degrade cell wall components (Azmir et al., 2013). In the juice industry, this leads to an increase in the juice yield, and in the extraction of bioactive compounds (Bender et al., 2017).

Spray-drying is an effective, low-cost method to dehydrate fruit juices, extending the shelf-life and reducing transportation costs, while allowing the encapsulation of bioactive compounds. Numerous studies analyze spray-drying of fruit juices in order to obtain physically stable powders: berries (Gagneten et al., 2019), blackcurrant (Archaina et al., 2018), jamun fruit (Santhalakshmy et al., 2015), among others.

Jelly desserts are traditionally produced from gelatin, water, flavorings, and dyes, being color one of the most important attributes for the product's acceptance (Sigurdson, et al., 2017). These desserts are usually formulated using artificial colorants, and they do not provide any bioactive compounds. Therefore, developing a nutritionally richer dessert with no artificial colorants can help improve diet quality. In this context, the aims of this work were: 1) to optimize the extraction process of blackcurrant juice by means of an enzymatic treatment and successive extraction cycles to maximize the recovery of bioactive compounds and juice yield; 2) to generate a blackcurrant-powder ingredient by spray-drying; and 3) to test the blackcurrant-powder as a potential natural colorant in a jelly dessert.

2. Materials and methods

2.1. Materials

Frozen blackcurrants (*Ribes nigrum*, Titania cultivar) and unflavored gelatin were purchased in a local market. Maltodextrin (MD, DE12) was from Givaudan S.A. (Buenos Aires, Argentina). Pectinex® Ultra Color (pectin-lyase enzyme) was from Novozymes (Bagsvaerd, Denmark).

2.2. Sample preparation

Fruits were thawed in a water bath at 40°C for 30 min, and ground with a manual grinder for 90 seconds to obtain a pulp.

2.3. Enzymatic treatment

To optimize the extraction conditions, an experimental design using a three-level and three-factors Box–Behnken Design (BBD) was applied (**Table 1**). The independent variables were: enzyme concentration (100-200 ppm), incubation time (20-60 min) and incubation temperature (35-45°C). Response variables were: juice yield (%), total phenolic compounds (TPC) and antioxidant capacity (AC).

The enzyme was added to the ground fruits and the mixture was incubated at the corresponding conditions (**Table 1**). Then, the enzyme was inactivated at 70°C, the mixture was cooled, and centrifuged for 20 min at 9700 g at 5°C in a Sorvall RC5C centrifuge (Sorvall Instruments, Wilmington, USA). Juice yield was calculated as the ratio between the original sample weight and the obtained juice.

For the optimization procedure, response surface methodology along with the desirability function was employed in order to maximize the response variables, and achieve the optimal extraction conditions. A whole duplicate of the complete design was carried out. Finally, two controls were assessed: a) *Control T*: an extraction conducted at the optimal conditions but omitting the enzyme; b) *Control*: an extract prepared at 25°C but omitting the enzyme.

Three-step successive extractions were carried out over the fruit under the optimal conditions and the supernatants from each cycle were merged to obtain one single *Optimal* juice.

2.4. Spray-drying

The *Optimal* juice was mixed with 20% w/w maltodextrin and spray-dried in a Mini Spray Dryer Büchi B290 (Flawil, Switzerland). Settings: inlet-air temperature 170±3°C, flow rate 8mL/min, air pressure 3.2 bar and 1.5 mm nozzle diameter, according to Gagneten et al. (2019).

2.5. Jelly dessert preparation

The dessert was formulated by mixing gelatin and the blackcurrant powder in a 1:3 ratio. This mixture was dissolved in boiling water (1:10 powder:water), the components were dissolved instantly. The preparation was poured into molds and stored at 4°C.

2.6. Physical Properties

All the determinations were performed in triplicate and the average results and standard deviations were informed.

2.6.1. Moisture content

It was determined by Karl Fischer titration with a TIM 980 Titration Manager (Villeurbanne Cedex, France). Methanol was used as solvent and Hydranal Titrant Composite 5 from Riedel-de Haën (Germany) as titration reagent.

2.6.2. Water activity (a_w)

It was analyzed by an electronic dew point water activity meter Aqualab Series 3TE (Decagon Devices, Pullman, Washington, USA).

2.6.3. Water sorption isotherm

It was determined by Dynamic Vapor Sorption (Intrinsic DVS, Surface Measurement Systems, UK) according to Enrione et al. (2007). For details see **Supporting information (Methods S1)**.

2.6.4. Glass transition temperature (T_g)

It was evaluated using differential scanning calorimetry in a DSC 822e Mettler Toledo calorimeter (Schwerzenbach, Switzerland). For details see **Supporting information (Methods S2)**.

2.6.5. Solubility and hygroscopicity

Both variables were determined according to Archaina et al. (2018). Solubility was expressed as percentage and hygroscopicity as grams of adsorbed water per 100 grams of dry matter (g a.w./100g d.m.). For details see **Supporting information (Methods S3)**.

2.7. Chemical and functional properties

A Jasco V-630 ultraviolet-visible (Jasco International Co., Tokyo, Japan) was used for spectrophotometric determinations. Powders were previously reconstituted by dissolving 0.50 g of powder in 25 mL distilled water. The pH was determined with a Mettler Toledo Seven Easy pH meter (Mettler Toledo, Switzerland).

2.7.1. Monomeric anthocyanin content (ACY)

Ethanolic extracts of the samples were prepared, and ACY was determined using the pH differential method according to Gagneten et al. (2019). ACY was expressed as cyanidin-3-glucoside per 1.00 g sample. For details see **Supporting information (Methods S4)**.

2.7.2. Total phenolic content (TPC) and antioxidant capacity (AC) by ABTS radical cation bleaching assay

Methanolic extracts of the samples were prepared and TPC (Folin-Ciocalteu method) and AC (bleaching method of the radical cation $ABTS^{+\bullet}$) were determined according to Gagneten et al. (2019). Calibration curves were prepared with gallic acid standard and the results were expressed as gallic acid equivalents in milligrams per 1.00 g sample. For details see **Supporting information (Methods S5)**.

2.8. Jelly dessert shelf-life study

The following variables were studied during 15 days at 4°C:

2.8.1. Superficial color

A handheld colorimeter (Minolta Co, CM-700d, Japan) was used. Color functions were determined for illuminant C at 2° standard observer. The global color difference function (ΔE^*_{00}) was calculated according to Luo et al. (2001). For details see **Supporting information (Methods S6)**.

2.8.2. Syneresis analysis

The syneresis magnitude was evaluated by measuring the volume of spontaneous gel exudation, and was expressed as percentage of the total gel mass. Three control samples were analyzed: 1) *Control-P*: jelly formulation without blackcurrant powder; 2) *Control-pH:* jelly formulation without blackcurrant powder at pH= 3.67±0.00; and 3) *Control-MD:* jelly formulation with maltodextrin but no fruit powder.

2.8.3. Word association task

This test was used to reveal consumer's perception of the jelly dessert (Ares et al., 2008). Participants were given a poll through social networks (**Figure 1**). The responses were evaluated using the Word Art web-based free service to create a word cloud. The words were grouped into different categories and their frequency of appearance was determined. Also, a 5-point hedonic scale was used to evaluate the consumers' acceptance of the product, including a question to analyze the purchase intent.

2.9. Statistical analysis

Statgraphics Centurion XVI statistical software (Virginia, USA, 2009) was used for the experimental design, data analysis and response surface graphs. Statistical analysis was performed using Graph Pad Prism 6 (California, USA, 2014). ANOVA analysis and the Tukey's test were carried out to detect significant differences ($p<0.05$).

3. Results and discussion

3.1. Optimization of extraction

The first objective of this work was to pursue an exhaustive extraction of blackcurrant juice; in this sense an enzymatic pretreatment was applied to the berries in order to improve the extraction of juice and bioactive compounds. Enzymatic action helps separate the pectins from the cell wall and degrades the soluble pectins, favoring the release of the soluble components (McLellan and Padilla-Zakour, 2004).

In order to perform the extraction optimization, a Box–Behnken Design (BBD) was applied. **Table 1** shows the responses (juice yield, AC and TPC) values achieved at the different conditions defined by the BBD. The regression model was predicted by equations (1), (2) and (3):

$$Y^{Yield} = 57.4033 + 1.24437\,X1 + 3.45063\,X2 + 1.64125\,X3 + 1.20396\,X1^2 - 1.17625\,X1\,X2 - 0.635\,X1X3 + 0.116458\,X2^2 + 0.2625\,X2X3 + 0.740208\,X3^2 \qquad (1)$$

$$Y^{AC} = 1.74117 + 0.02025\,X1 + 0.112437X2 + 0.0435625X3 - 0.0650208X1^2 - 0.17675X1\,X2 + 0.09625\,X1X3 - 0.106896\,X2^2 + 0.020625\,X2X3 + 0.0793542X3^2 \qquad (2)$$

$$Y^{TPC} = 5.94167 + 0.151938\,X1 + 0.36475X2 + 0.169688\,X3 + 0.104417\,X1^2 + 0.039375X1\,X2 + 0.16225\,X1X3 - 0.0219583\,X2^2 + 0.065375X2X3 + 0.0476667X3^2 \qquad (3)$$

Where, X_1 = temperature, X_2 = time and X_3 = enzyme concentration.

Figure 2 shows a Pareto chart presenting the effect of each independent variable on the responses. The three variables significantly contributed to the increase of the juice yield. However, none of the variables had a significant contribution for AC, while the treatment time and the enzyme concentration significantly contributed to TPC rise. Noteworthy, the incubation time was the most significant variable in the process, followed by the enzyme concentration and finally, the temperature. Regarding the interactions between variables, only temperature-time had a significant contribution for yield response (p = 0.03).

Other authors obtained similar results by enzymatic maceration of blackcurrant, maximizing the extraction of bioactive compounds (Bagger-Jørgensen and Meyer, 2004), and obtaining better juice yields (Bender et al., 2017).

The optimal treatment conditions predicted by the design were 200 ppm of enzyme, 45°C and 60 min. The desirability function reached a value of 0.8772, which indicates that a good maximization was achieved.

The juice yield in the optimal treatment was 65%, showing a 15% increase compared to the control. Additionally, the influence of the enzyme and the temperature under the optimal conditions were studied (**Figure 3**). The increase of ACY, TPC and AC in the optimal juice was of 2.7, 2.4 and 2.8 times, respectively compared to the *Control*. In contrast, the increase with respect to *Control T* was of 1.8, 1.7 and 1.5 times for the three responses, respectively. Comparing the results for both controls, a significant effect of temperature on bioactive compounds extraction was observed. Cacace and Mazza (2002) reported an increase of the rate of diffusion of bioactive compounds in blackcurrant with increasing temperature.

These findings suggest that the high recovery of bioactive compounds due to enzyme action is the result, not only of the enhancement of the juice yield, but also of the significant improvement on the release of bioactive compounds from the fruit.

A three-step extraction procedure was performed following the optimal conditions. The second and third extraction cycles showed a 40% and 20% recovery compared to the first one, showing that a single-step extraction was not sufficient to recover most of blackcurrant's bioactive potential.

3.2. Blackcurrant-powder development and characterization

A blackcurrant-powder was generated by spray-drying the *Optimal* juice. The powder presented relatively good physical properties, adequate to be stored in a hermetic container at room temperature: low moisture content (3.40±0.03g H_2O/100g d.b.), low a_w (0.130±0.001), and a T_g value above room temperature (40±1°C).

Figure 4 shows the water sorption isotherm (20^{o}C) for the blackcurrant powder. The shape of the isotherm followed a type III sorption behavior, typical of foods with high proportion of sugars. Experimental data were fitted to Peleg´s equation (R^2= 0.998), and the adjusted parameters were k_1:12.3%, K_2: 55.9%, n_1: 1.0 and n_2: 4.03. Červenka (2011) concluded that Peleg's model was the best for describing water sorption data of berries.

Regarding hygroscopicity, a value of 18.3±0.3 g a.w./100g d.m. was obtained. High values may affect the shelf life of powders since a small increase in the water content can cause a decrease of T_g and reduce its stability (Roos, 2002). Other authors obtained hygroscopicity values of 11.8 g a.w./100g d.m. (Gagneten et al., 2019) and 14.46 g a.w./100g d.m. (Archaina et al., 2018) for spray-dried blackcurrant extracts. Santhalakshmy et al. (2015) found hygroscopicity values between 17.00 and 25.33 g a.w./100g d.m. for spray-dried jamun juice.

A high solubility is important to facilitate the incorporation of the powder in the food matrix. In this work a solubility value of 96.1±0.1% was obtained. Santhalakshmy et al. (2015), obtained solubility values between 88 and 100% for jamun powder. Archaina et al. (2018) and Gagneten et al. (2019) obtained solubility values around 94% for blackcurrant powders, although with higher concentrations of MD.

Regarding the functional properties, the obtained values were: TPC: 8.6±0.5 mg GA/g; ACY: 4.2±0.1 mg cyd-3-glu/g; and AC: 4.63±0.07 mg GA/g. These values were between 3 and 5 times higher than those obtained for blackcurrant powder obtained using ultrasound assisted extraction (Gagneten et al., 2019; Archaina et al., 2018).

Overall, the obtained blackcurrant-powder showed satisfactory physical properties together with interesting bioactive compounds content, to be considered as an ingredient to be incorporated into desserts.

3.3. Blackcurrant-powder application in a jelly dessert: characteristics and stability

The blackcurrant-powder was evaluated as an ingredient in a jelly dessert. The formulated jelly presented the following functional input: TPC= 0.56±0.03 mg GA/g, ACY= 0.20±0.02 mg cyd-3-

glu/g and AC= 0.16±0.02 mg GA/g. The pH of the final product was 3.67±0.01. When comparing the TPC contribution of this dessert with that of fresh fruits, it was found that one portion of the jelly (100 g) would provide, for instance, half the content of one pear (1.08 mg GA/g) (Navarro González et al., 2017).

Home-prepared jelly desserts' shelf-life at refrigeration temperature is generally lower than two weeks. Therefore, the stability of the jelly was followed during 15 days at 4°C. The chromatic coordinates for the jelly at the beginning of the storage were: L*= 5.4±0.7, a*= 4.3±0.2, and b*= -1.6±0.4, which correspond to a very dark red color. After 15 days of storage the chromatic variables were: L*= 3.4±0.3, a*= 5.2±0.3, and b*= 0.8±0.1. The color changes along storage indicate a slight darkening, a slight decrease in the red component, and also, an increase in b*, shifting from the blue to the yellow quadrant, probably related to the development of browning. **Figure 5** shows the global color difference (ΔE^*_{00}) during storage at 4°C. Keraitė et al. (2017) established a useful classification of ΔE^*_{00} values to correlate the instrumental measurement of color with the perception of the human eye. Until day 12 the color difference would be only perceptible by close observation ($1<\Delta E^*_{00}<2$) and at the end of storage, it would be perceptible at a glance ($2<\Delta E^*_{00}<10$).

Regarding functional properties, no significant differences were detected at the end of storage (data not shown). The phytochemicals present in blackcurrant showed high stability at low pH values (Zhao, 2007).

Syneresis is a critical factor in jelly desserts regarding consumers' acceptance, therefore, this phenomenon was analyzed during storage. *Control-P* sample presented 12±3% syneresis at the end of the storage period, while the jelly dessert, *Control-MD*, and *Control-pH* samples did not show significant syneresis. Mahdavi et al. (2016) formulated a jelly with barberry-juice powder using different carriers and found that regardless the used matrix, the syneresis decreased with respect to a control without fruit powder after 30 days storage at 25°C. Clearly, the presence of a matrix like maltodextrin and the low pH had a positive effect preventing syneresis in the jelly dessert.

3.4. Qualitative sensory study on the jelly dessert

The word association task was applied to explore the consumer's perception about the product. **Figure 6** shows a cloud of words with the 212 responses obtained from the test. These words were grouped in seven categories, which showed the following frequencies of mention: a) *Healthy*: 24.1%; b) *Interesting*: 21.7%; c) *Liking expressions*: 17.0%; d) *Natural*: 12.7%; e) *Innovative*: 11.3%; f) *Other*: 6.1%; g) *Intention to try it*: 5.2%; and h) *Negative responses*: 1.9%. *Other* responses included words such as "unknown", "fruit", "perplexed". Mostly positive associations were obtained (92.9%).

Regarding the question "how much do you like this product?", 81.0% of people affirmed to like the product ("Like" and "Like very much"), while only 3.3% of people gave disliking answers ("Dislike" and "Dislike very much"). The percentage of "Neither like nor dislike" responses was 15.3%. Concerning the question about purchase intention, 73.5% answered that they would buy it, 20.9% that they might buy it, and only 5.6% answered negatively.

4. Conclusions

The enzymatic treatment improved the blackcurrant juice by increasing the juice yield, and enhancing the extraction of bioactive compounds from the fruit. Time, temperature and enzyme concentration showed a positive effect over the bioactive compounds recovery and the AC, being the treatment time the most influencing parameter. Moreover, the use of successive extraction steps was needed in order to maximize the bioactive compounds extraction.

A blackcurrant powder with relatively good physical characteristics and potential functional properties was obtained. The use of this powder as a natural colorant in a jelly dessert showed promising results. The ingredient not only enriched the product with bioactive components, but also provided a stable and attractive color. Moreover, the gel properties were improved and the jelly showed very good acceptance in the consumer's perception test. These results prove that this ingredient has good potential to be used as natural colorant and functional additive in food.

5. Acknowledgments

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Legends for figures

Figure 1. Word association task form.

Figure 2. Pareto Charts: effect and significance of the independent variables: time, enzyme concentration, and temperature on the responses (a) Yield, (b) AC and (c) TPC.

Figure 3. Bioactive compounds content and AC obtained in the first extraction cycle for the *Optimal* and for the *Control* and *Control T* procedures. a) ACY, b) TPC and c) AC. Bars with a different lowercase letter are significantly different ($p<0.05$).

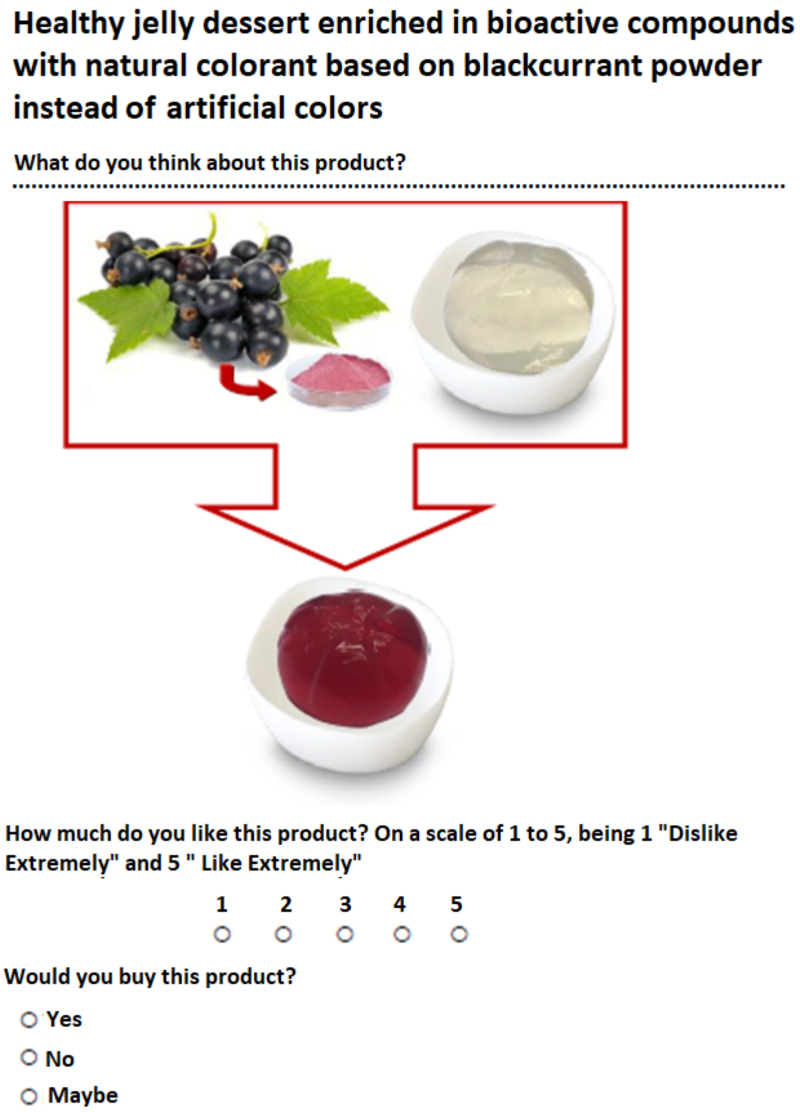
Figure 4. Water sorption isotherm at 20°C for blackcurrant spray-dried powder (Experimental data: diamonds). The continuous curve corresponds to values predicted by Peleg´s model.

Figure 5. Global color change (ΔE^*_{00}) for jelly dessert during storage at 4°C.

Figure 6. Cloud of words constructed with the results from the qualitative sensory test.

Table 1. Experimental design used to optimize the extraction conditions and results for the response variables along with their correspondent standard deviations.

	Factor values			Response values		
Number	Temperature (°C)	Enzyme concentration (ppm)	time (min)	Yield (%)	AC (mg GA/ g extract)	TPC (mg GA/ g extract)
1	35	100	40	55.0±0.9	1.52±0.08	5.80±0.13
2	35	150	20	52.7±1.7	1.14±0.11	5.13±0.13
3	35	150	60	62.7±1.9	1.86±0.05	6.07±0.07
4	35	200	40	60.7±1.8	1.71±0.16	5.82±0.27
5	40	100	20	54.4±0.5	1.48±0.12	5.41±0.15
6	40	100	60	60.1±2.3	1.92±0.12	5.81±0.27
7	40	150	40	57.9±0.3	1.50±0.10	5.12±0.35
8	40	150	40	56.6±1.5	1.63±0.08	5.68±0.37
9	40	150	40	57.7±0.2	1.90±0.11	6.37±0.45
10	40	200	20	56.0±0.1	1.64±0.06	5.64±0.28
11	40	200	60	62.7±0.1	2.07±0.14	6.42±0.26
12	45	100	40	59.2±1.7	1.99±0.07	6.25±0.06
13	45	150	20	57.1±0.9	1.74±0.05	6.04±0.10
14	45	150	60	62.4±3.2	1.97±0.08	6.64±0.19
15	45	200	40	64.4±0.6	2.17±0.03	6.67±0.19



Healthy jelly dessert enriched in bioactive compounds with natural colorant based on blackcurrant powder instead of artificial colors

What do you think about this product?

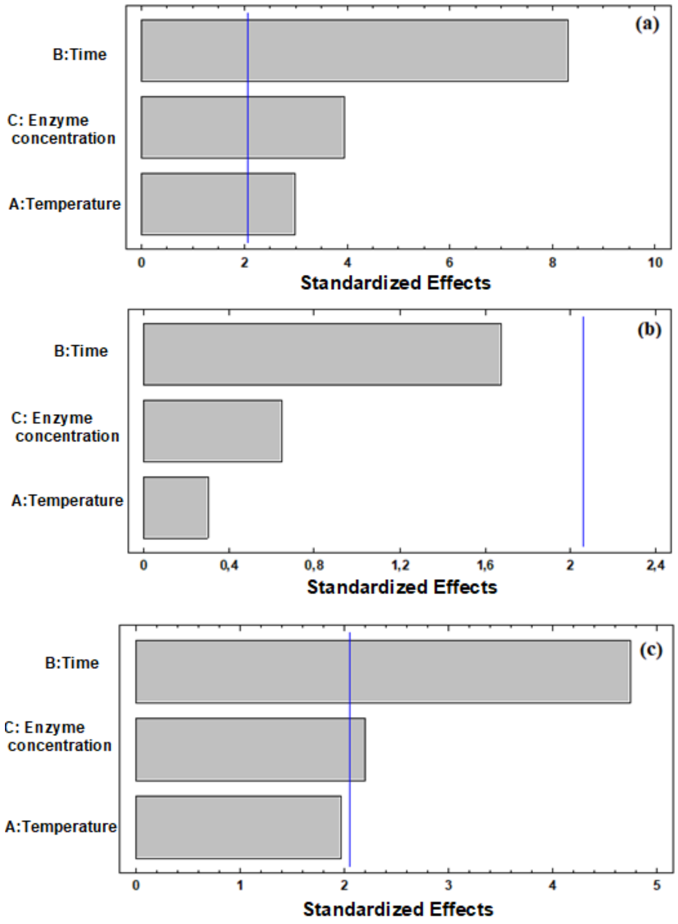
How much do you like this product? On a scale of 1 to 5, being 1 "Dislike Extremely" and 5 " Like Extremely"

1	2	3	4	5
○	○	○	○	○

Would you buy this product?

○ **Yes**
○ **No**
○ **Maybe**

jfpp_15011_f1.tif



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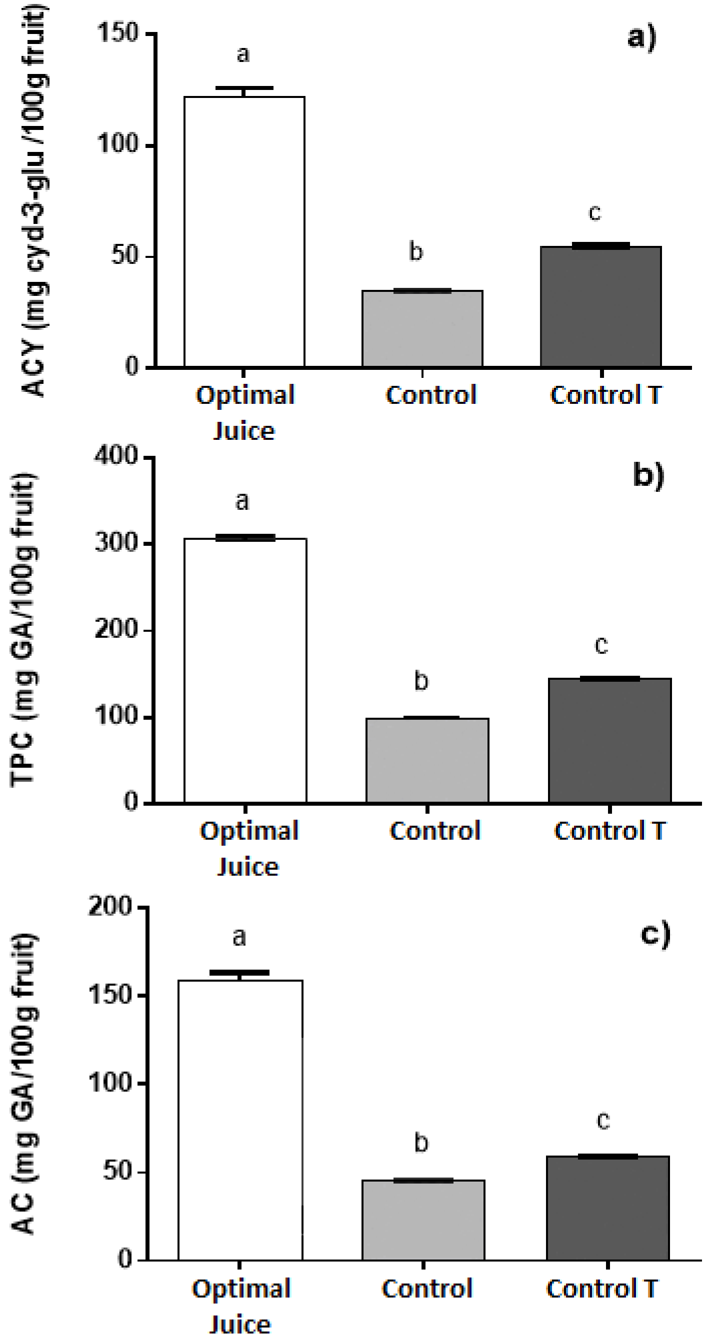

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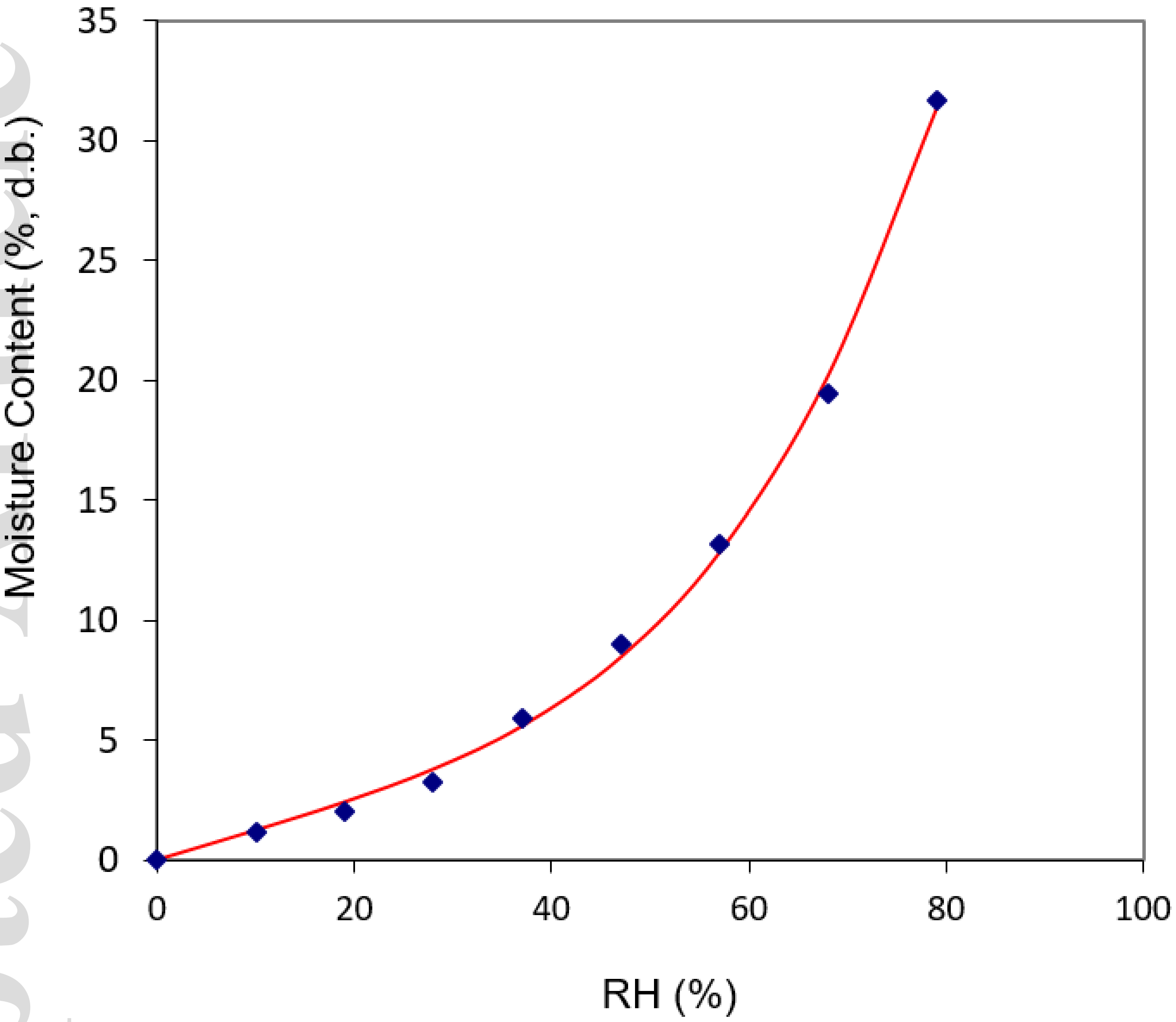

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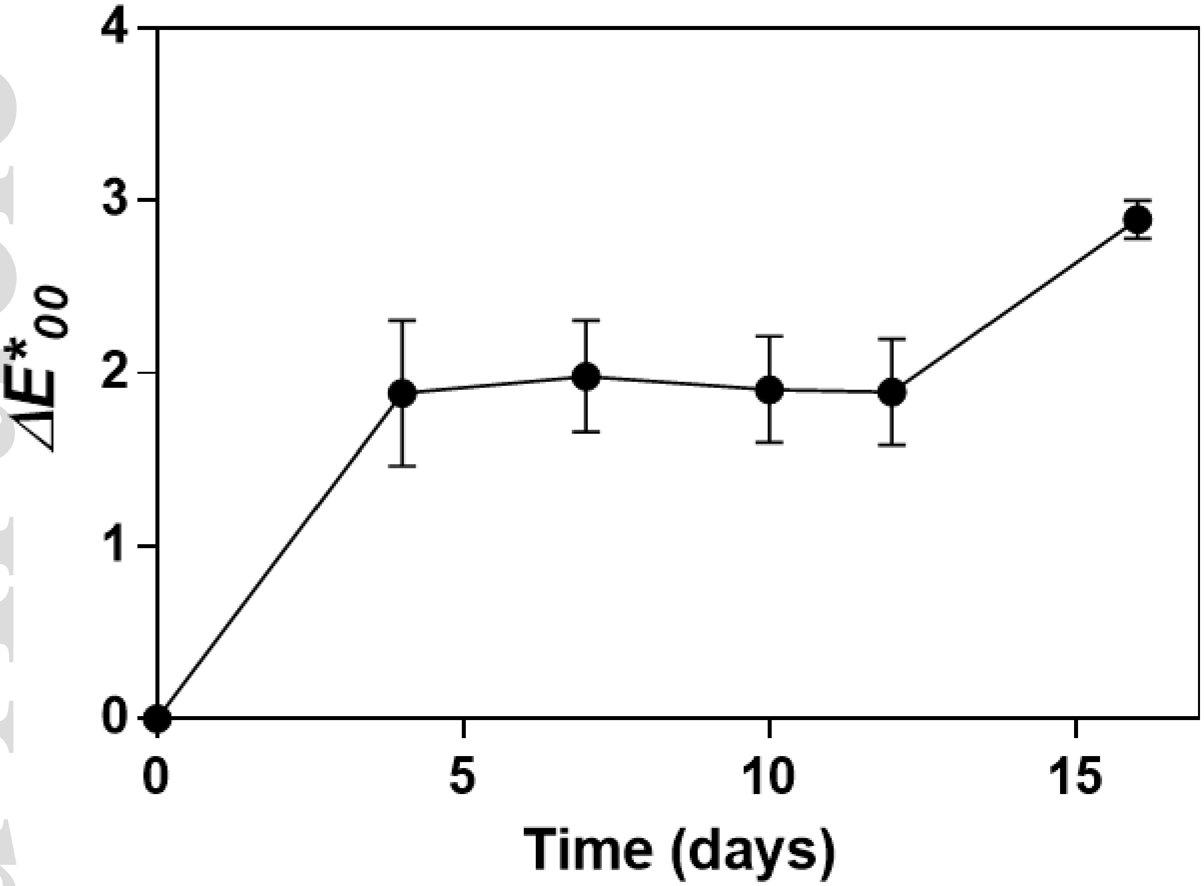

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