R E S E A R C H   A R T I C L E

New flavour bars with cherry, almond and honey

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A B S T R A C T

Snacks are commonly eaten in western societies and, because of that, have a non-negligible effect on consumers' health. The main
objective of this work was to develop and optimize a snack formulation with sweet cherry, an important crop from the Portuguese region
of Beira Interior and which has a Protected Geographical Indication (PGI). The experimental design and the optimization process were
based on the Response Surface Methodology. In order to achieve that, a factorial design was implemented with three factors (almond and
honey content, and baking time) and five response variables (water activity, antioxidant activity, mesophilic count, flavour and texture),
and three repetitions of the central point. The optimization resulted in a formulation with 50 g 100 g^{-1} of sweet cherry, 35 g 100 g^{-1} of
almond, 15 g 100 g^{-1} of honey, baked in the oven at 120 ºC for 13 min. The nutritional analysis showed that this product could be labelled
with some nutritional claims, such as "low saturated fat", "with no added sugar", "salt free" and "source of fibre".

Keywords: Almond; Honey; Response surface methodology; Snack, Sweet cherry

INTRODUCTION

The word "snack" is used, primarily, to designate a portion of a solid food or beverage, smaller than a main meal, and eaten or drunk between main meals. In fact, it is a broad word which can be used for several types of food products, fresh, processed or prepared at home. In these kinds of products, we have the snack-bars (Gatenby, 1997; Chaplin and Smith, 2011).

Snack consumption is broadly dispersed among western societies. For instance, a study implemented in UK in 2004 revealed that 90 % of the respondents regularly ate snacks and an average of 410 snacks were consumed per person per year (Lloyd-Williams et al., 2008). Additionally, food ingestion between main meals could represent 25 % of the total energy intake (Kant and Graubard, 2015). Because of that, snacking has a non-negligible effect on health, which could be beneficial or harmful depending on the ingredients and on the preparation method (Bucher et al., 2016; Green et al., 2017).

Sweet cherry (*Prunus avium* L.) is a major crop in the Portuguese Beira Interior region with a Protected Geographical Indication (PGI) (EC, 1996). Like most fresh fruits, sweet cherry is a dietary source of antioxidant compounds linked with the reduction of the incidence of oxidative related diseases like cancer and vascular diseases. Additionally, it could improve sleep, cognitive functions and help to relieve muscular pain after hard training (Usenik et al., 2008; Liu et al., 2011; Prvulovic et al., 2011; Kelley et al., 2018). The antioxidant properties of this fruit come essentially from polyphenol compounds, melatonin, carotenoids and vitamins C and E. They are highly variable, depending on several factors such as cultivars, ripening stage, edaphoclimatic conditions, and cultural and post-harvest practices. In fact, the antioxidant activity of sweet cherry could be as low as 3.7%DPPH (1.1-diphenyl-2-picrylhydrazyl) reduction or as high as 44.3%DPPH reduction (Prvulovic et al., 2011; Kelley et al., 2018).

Almond (*Prunus dulcis* (Mill.) D. A. Webb) is also a source of antioxidant compounds (like polyphenols and α-tocopherol), high digestible proteins and unsaturated

fatty acids. It is linked with the reduction of the cholesterol levels, glycemic regulation and with some anti-inflammatory properties (Kamil and Chen, 2012). Almond has also a beneficial influence on the intestinal microorganisms and in colon cancer prevention (Mandalari et al., 2008). Kiat el al. (2014) mentioned 47.7%DPPH reduction for the antioxidant activity of almonds.

Honey has been used as a sweetener, analgesic, anti-inflammatory and antiseptic for millennia (Ferreira et al., 2009). The antiseptic properties of honey come mainly from hydrogen peroxide and from minerals like copper and iron (Ferreira et al., 2009). The antioxidant compounds from honey are essentially enzymes, like glucose-oxidase and peroxidase; vitamins, carotenoids and flavonoids (Ferreira et al., 2009). Those compounds are related with health benefits, like the protection from oxygen reactive species (Chua et al., 2013). The values found for the antioxidant activity of honey varied from 35.7 to 86.9%DPPH reduction (Baltrusaityte et al., 2007; Escuredo et al., 2013).

Response Surface Methodology (RSM) has been used as a tool for the optimization of different categories of food products, including snacks. The factors chosen to be optimized are usually related with the composition and/or preparation of the product, namely, the oven temperature and baking time (Nath and Chattopadhyay, 2007; potato-soy snacks), the levels of the ingredients, the oven temperature and the baking time (Jan et al., 2018; quinoa snacks), temperature, time, water/tea ratio and particle size (Liu et al., 2018; green tea infusion) and the steaming time, gel setting time and drying temperature (Ramesh et al., 2018; fish crackers). The response variables are mostly related with the chemical/nutritional content (Nath and Chattophadyay, 2007; Jan et al., 2018; Liu et al., 2018), physical/mechanical properties (Pardhi et al., 2017; Jan et al., 2018; Ramesh et al., 2018) and sensory characteristics (Nath and Chattopadhyay, 2007; Jan et al., 2018; Liu et al., 2018).

The main objective of this work was to optimize a snack-bar formulation based exclusively in sweet cherry, almond and honey. More specifically, it was intended to find the best proportions between the ingredients and the best baking time in order to obtain a product with healthy properties, microbiological stability and consumer acceptance, while simultaneously promoting the valorisation of Portuguese regional products.

MATERIALS AND METHODS

Raw materials and preparation
The snacks were formulated exclusively with dehydrated sweet cherry (*Prunus avium* 'Satin'; 14.8% of moisture content), almond (*Prunus dulcis* 'Ferraduel') and honey. Prior to the preparation, the almonds were toasted in the oven at 180ºC for 15 min. The ingredients were ground, kneaded manually and put in a silicon baking container (5.4 x 2.8 x 1.2 cm). All the formulations were cooked in the oven at 120ºC. Three independent batches were prepared for each formulation.

Experimental design
The formulation optimization was carried out through a factorial design with 3 repetitions of the central point and 3 factors: almond content (g 100 g^{-1}) (X_1), honey content (g 100 g^{-1}) (X_2) and baking time (min) (X_3), each one with 3 levels, corresponding to 5, 20 and 35 g 100 g^{-1} for almond content; 5, 10 and 15 g 100 g^{-1} for honey content and 7, 10 and 13 min for baking time.

The experiment consisted in a total of 11 formulations (Fig. 1) with 3 repetitions of the central point (formulations F09, F10 and F11) (Table 1) and 5 response variables: water activity (Y_1), antioxidant activity (Y_2), mesophilic count (Y_3), Flavour (Y_4) and Texture (Y_5).

Qualitative evaluation of the formulations
The water activity was determined at 25ºC using the equipment HygroPalm (HP 23, Rotronic).

The antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) as free radical, according to Brand-Williams et al. (1995). The absorbance was determined in the spectrophotometer Cintra 202 (GBC) and the results were expressed as the percentage of DPPH reduction.

The aerobic mesophilic microorganisms count was evaluated according to ISO 4833-1:2013 using Plate Count Agar as culture medium. The results were expressed as Colony Forming Units (CFU) g^1 and transformed in log$_{10}$ CFU g^{-1}.

The hedonic evaluation of Flavour and Texture was carried out by 10 non-trained panellists with ages between 23 and 49 in a Sensory Laboratory equipped according to ISO 8589:2007/Amd 1:2014. The samples were presented randomly in Petri dishes, coded with a three digits number. The panellists were asked to rinse their palate with water after the evaluation of each sample. Flavour and Texture were evaluated in a hedonic 9-points scale (1- Dislike extremely, 5- Neither like nor dislike, 9- Like extremely). A minimum classification of 5.0 was defined as an acceptance indicator.

The energetic value and the nutritional composition were determined for the optimal formulation. Energetic value
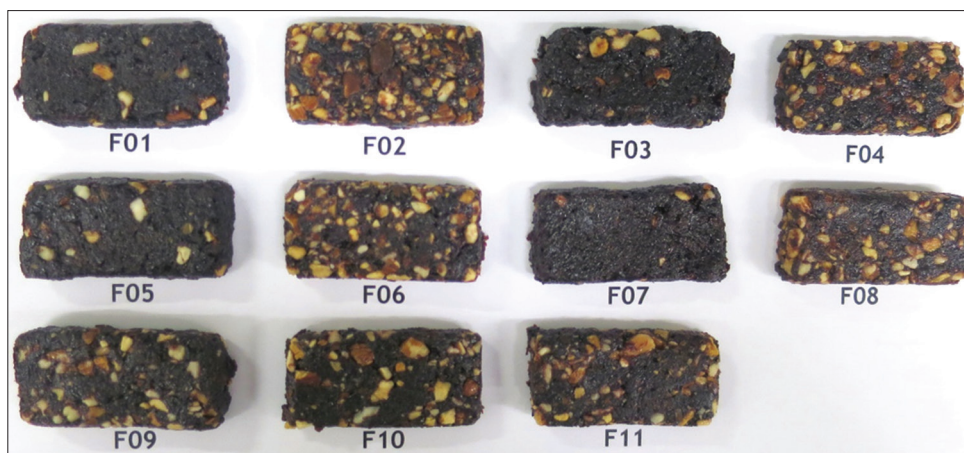
Fig 1. Formulations (F01 to F11) used for the optimization of the sweet cherry, almond and honey snack-bar carried out through a factorial design with 3 factors (almond content, honey content and baking time) and 3 repetitions of the central point (formulations F09, F10 and F11). The composition (almond and honey content) and the baking time for each formulation were, by that order, the following: F01- 5 g 100 g^{-1}, 5 g 100 g^{-1} and 7 min; F02- 35 g 100 g^{-1}, 5 g 100 g^{-1} and 7 min; F03- 5 g 100 g^{-1}, 15 g 100 g^{-1} and 7 min; F04- 35 g 100 g^{-1}, 15 g 100 g^{-1} and 7 min; F05- 5 g 100 g^{-1}, 5 g 100 g^{-1} and 13 min; F06- 35 g 100 g^{-1}, 5 g 100 g^{-1} and 13 min; F07- 5 g 100 g^{-1}, 15 g 100 g^{-1} and 13 min; F08- 35 g 100 g^{-1}, 15 g 100 g^{-1} and 13 min; F09, F10 and F11- 20 g 100 g^{-1}, 10 g 100 g^{-1} and 10 min. The sweet cherry content was the difference between 100 g and the combined content of almond and honey.

Table 1: Decoded matrix of the factorial design of the sweet cherry, almond and honey snack-bars with 3 repetitions of the central point (formulations 9 to 11) and 3 levels for each factor

Formulation	Sweet cherry content (g 100 g^{-1})	X_1	X_2	X_3
		Almond content (g 100 g^{-1})	Honey content (g 100 g^{-1})	Baking time (min)
F01	90	5	5	7
F02	60	35	5	7
F03	80	5	15	7
F04	50	35	15	7
F05	90	5	5	13
F06	60	35	5	13
F07	80	5	15	13
F08	50	35	15	13
F09	70	20	10	10
F10	70	20	10	10
F11	70	20	10	10

calculation was based on lipid, carbohydrates, protein and fibre content (EU, 2011). The results were expressed in kJ and kcal per 100 g of product and per dose (18 g).

The ashes, protein and lipid content were determined by the method described by AOAC (2000) and the fibre content was determined by the method described by AOAC (1990). Results were expressed as g 100 g^{-1}. Carbohydrates content was calculated by difference after the determination of moisture, lipids, fibre, protein and ashes content (EU, 2011).

Saturated fatty acids fraction was quantified after lipid extraction, followed by basic methylation, identification and quantification by gas chromatography coupled with a flame ionization detector (GC-FID 7890 A Agilent Technologies). The DB-WAXetr column (30 m, 0.25 mm, 0.25 μm), the 7683 B injector were used (split mode), at 260°C.

For the sugar fraction determination, firstly the interferents were removed through a solid phase extraction column (SPE-C18, 500 mg 10 mL^{-1}). The sugars (glucose, fructose, sucrose, lactose and maltose) were quantified by ionic chromatography (ICS-3000, Dionex) with an electrochemical detector and the Chromeleon software. The column CarboPac PA20 (3x150 mm), the pre-column CarboPacGuard (3x30 mm), a flow rate of 0.5 mL min^{-1} and 2 mobile phases (200 mM and 15 mM NaOH) were used. The results were expressed as g 100 g^{-1}.

Sodium was quantified after the sample microwave digestion (Ethos One, Milestone) by atomic absorption

spectroscopy (ICE3000 Thermo Scientific) at 589 nm. The results were expressed as mg 100 g^{-1}.

Data analysis

The mean and the standard error of the three independent batches were determined for each response variable. One-way analysis of variance (ANOVA) and Tukey multiple comparisons post-hoc test were carried out to determine the significant differences (p<0.05) between means, using the software IBM SPSS 21.

For the Response Surface Methodology (software Minitab 18), a 2nd order polynomial model was adjusted to each response variable, as shown in Equation 1.

$$Y_k = \beta_{k0} + \sum_{(i=1)}^{3} \beta_{ki}.X_i + \sum_{(i=1)}^{3} \beta_{kii}.X_i^2 + \sum_{(i \neq j=1)}^{3} \beta_{kij}.X_i.X_j$$
Equation 1

Where Y_k represents the response variable, X_i and X_j represent the factors, β_{k0} represents the intercept, β_{ki} represents the linear coefficients, β_{kii} represents the quadratic coefficients and β_{kij} represents the interaction coefficients.

The optimization was done through the maximization of the desirability function, considering the maximization of the response variables antioxidant activity, Flavour and Texture and the minimization of the response variables water activity and mesophilic count.

RESULTS AND DISCUSSION

Quality of the ingredients

All the ingredients showed a water activity lower than 0.6 (Table 2), which indicates the microbiological stability of the product (Tapia et al., 2007; Guiné et al., 2014). The water activity value for the dehydrated sweet cherry (0.495) was lower than what was described by Safe Food 360º (2014). For the almond, the water activity (0.491) was similar to what was described by Du et al. (2010) and by Guiné et al. (2014). The water activity found in honey (0.576) was lower than the value described by Safe Food 360º (2014) and similar to what was referred by Olaitan et al. (2007).

The antioxidant activity of the dehydrated sweet cherry (38.4%DPPH reduction) was similar to what was found by Prvulovic et al. (2011). Nevertheless, the antioxidant activity values found for almond and honey (19.8 and 5.9%DPPH reduction) were lower than those described previously in the literature (Baltrusaityte et al., 2007; Escuredo et al., 2013; Kiat et al., 2014).

The mesophilic count indicated a satisfactory quality for the honey (<1 log), dehydrated sweet cherry (2.4 log) and almond (3.4 log), according to what was defined by Gilbert et al. (2000).

Effect of the factors in the quality of the formulations

Table 3 shows the mean values, the ANOVA and the Tukey post-hoc results for the response variables, for each formulation. The parameters associated with the

Table 2: Water activity, antioxidant activity and mesophilic count for the tested snack-bar ingredients, namely dehydrated sweet cherry, almond and honey

Ingredient	Water activity	Antioxidant activity (%DPPH)	Mesophilic count (log$_{10}$ CFU g^{-1})
Dehydrated sweet cherry	0.495±0.003	38.4±1.0	2.4±0.1
Almond	0.491±0.002	19.8±0.3	3.4±0.2
Honey	0.576±0.002	5.9±0.1	<1

Mean ± standard error. N=3

Table 3: Values of the factors and experimental results for the response variables from the sweet cherry, almond and honey snack-bar factorial design with 3 repetitions of the central point (formulations F09 to F11)

Cherrie formulations	Factors			Response variables				
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5
	Almond content (g 100 g^{-1})	Honey content (g 100 g^{-1})	Baking time (min)	Water activity	Antioxidant activity (%DPPH)	Mesophilic count (log$_{10}$ CFU gS^{-1})	Flavour	Texture
F01	5	5	7	0.508±0.014^a	43.3±7.9^a	2.4±0.10^a	5.0±0.6^a	4.5±0.4^{ab}
F02	35	5	7	0.495±0.010^a	37.4±2.5^a	2.5±0.03^a	6.9±0.5^a	6.8±0.4^a
F03	5	15	7	0.503±0.012^a	33.5±4.0^a	2.4±0.08^a	4.9±0.5^a	4.7±0.5^{ab}
F04	35	15	7	0.495±0.008^a	34.0±3.0^a	2.5±0.15^a	6.5±0.5^a	6.1±0.5^{ab}
F05	5	5	13	0.498±0.006^a	45.6±2.0^a	2.5±0.01^a	5.0±0.7^a	3.8±0.6^b
F06	35	5	13	0.479±0.013^a	44.0±1.8^a	2.3±0.07^a	6.0±0.5^a	5.9±0.6^{ab}
F07	5	15	13	0.487±0.010^a	45.3±11.2^a	2.3±0.13^a	4.8±0.6^a	4.0±0.5^b
F08	35	15	13	0.467±0.008^a	38.1±5.0^a	2.5±0.12^a	6.6±0.4^a	6.8±0.4^a
F09	20	10	10	0.481±0.013^a	38.7±7.5^a	2.6±0.14^a	6.1±0.6^a	6.1±0.5^{ab}
F10	20	10	10	0.486±0.012^a	42.5±3.2^a	2.5±0.06^a	6.0±0.6^a	5.3±0.7^{ab}
F11	20	10	10	0.486±0.006^a	40.0±4.4^a	2.6±0.01^a	6.2±0.6^a	6.2±0.5^{ab}

Results presented as mean±standard error. Different letters in the same column indicate statistical differences at *P*<0.05.

Table 4: Estimated regression coefficients from the second order polynomial model adjustment to each one of the response variables used for the optimization of the sweet cherry, almond and honey snack-bar

	Y_1	Y_2	Y_3	Y_4	Y_5
	Water activity	Antioxidant activity (%DPPH)	Mesophilic count ($\log_{10}$ CFU g^{-1})	Flavour	Texture
Constant	0.52***	40.90*	2.39***	5.30**	5.92*
X_1 Almond content	-0.0014*	0.011	0.028	0.13*	0.14
X_2 Honey content	0.00067	-1.1	-0.018	-0.094	-0.11
X_3 Baking time	-0.00042	0.65	-0.0013	-0.074	-0.27
X_{11}	0.000033*	-0.0011	-0.00063	-0.0017	-0.0024
X_{22}	ne	ne	ne	ne	ne
X_{33}	ne	ne	ne	ne	ne
X_{12}	0.000006	0.0013	0.00052	0.00083	-0.00033
X_{13}	-0.000050	-0.0098	-0.00057	-0.0019	0.0033
X_{23}	-0.00015	0.057	0.00050	0.0075	0.013
R_2	0.9858	0.8635	0.7674	0.9703	0.9302
Lack of fit	*ns*	*ns*	*ns*	*ns*	*ns*

ne – non estimated due to high collinearity with other factors; *ns* – non significant statistical differences ($p > 0.05$); *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

adjustment of the second order polynomial models to the experimental data are shown in Table 4.

The R² was higher than 0.7 and the lack of fit was not significant (p≥0.05) for the tested models, showing a good adjustment to the experimental data and a good ability of this models to represent them (Jan et al., 2018).

The mean values of the water activity are comprised between 0.467 (F08) and 0.508 (F01), which indicates conditions that inhibit microbial growth (Schmidt and Fontana, 2007; Safe Food 360º, 2014). The almond content (both linear and quadratic) was the only factor that showed a significant and negative effect (p<0.05) in water activity (Table 4).

There were no significant differences (p≥0.05) between the antioxidant activity of the different formulations. The mean values from this response variable were comprised between 33.5%DPPH reduction (F03) and 45.3%DPPH reduction (F07) (Table 3) and are not far from the antioxidant activity of the dehydrated sweet cherry (38.4%DPPH reduction), which was the main ingredient in all formulations.

Regarding mesophilic count, there were no statistical differences (p≥0.05) among formulations. The values found were between 2.3 log (F06 and F07) and 2.6 log (F09 and F11), highlighting a satisfactory microbial quality of product (Gilbert et al., 2000).

No statistical differences were found (p≥0.05) in the Flavour between formulations. The Flavour mean classifications were comprised between 4.8 (F07) and 6.9 (F02) (Table 3). The almond content had a significant positive effect (p<0.05) in Flavour (Table 4).

Texture was the only studied response variable with significant differences (p<0.05) between formulations. In

Table 5: Model validation: predicted and experimental values of the response variables from the optimization of the sweet cherry, almond and honey snack-bar

Response variable		Predicted	Experimental
Y_1 Water activity		0.467	0.467
Y_2 Antioxidant activity	(%DPPH reduction)	39.5	38.1
Y_3 Mesophilic count	($\log_{10}$ CFU g^{-1})	2.4	2.5
Y_4 Flavour	(points)	6.5	6.6
Y_5 Texture	(points)	6.6	6.8

fact, F02 and F08 showed higher Texture classifications (6.8 for both formulations) than F05 and F07 (3.8 and 4.0, respectively) (Table 3).

There were four formulations that had a lower classification than the minimum defined for sensorial acceptance (i.e. 5.0 points) in, at least, one of the two sensory descriptors (Flavour and Texture). Those formulations were the F01, with 4.5 points in Texture; F03, with 4.9 points in Flavour and 4.7 points in Texture; F05, with 3.8 points in Texture, and F07 with 4.8 points in Flavour and 4.0 points in Texture. Additionally, the lowest almond content (5 g 100 g^{-1}) appeared to be related to a lower Texture classification, which might be explained by the crunchy sensation given by the toasted almond. Moreover, the adjusted model has indicated that the almond content had a significant effect (p<0.05) in the water activity and in the Flavour.

The simultaneous optimization of the response variables, performed by the maximization of the desirability function, showed the following values for, respectively, the almond content, the honey content and the baking time at 120ºC: 35 g 100 g^{-1}; 15 g 100 g^{-1} and 13 min, which are identical to the F08 formulation. This result may indicate that, in a future attempt, the variation amplitude of the factors in study should be increased.

Table 6: Energy value and nutritional content of the optimized formulation of the sweet cherry, almond and honey snack-bar

	Units	Nutrient content [a]	
		100 g	Dose (18 g)
Energy value	kcal	430.4±4.3 (21.5%)	77.5±0.8 (3.9%)
	kJ	1810.2±17.1 (21.5%)	325.8±3.1 (3.9%)
Water	g	9.5±0.3	1.7±0.1
Fat	g	16.8±1.0 (24.0%)	3.0±0.2 (4.3%)
Saturated fatty acids	g	1.5±0.1 (7.5%)	0.27±0.02 (1.4%)
Carbohydrates	g	58.2±1.4 (18.2%)	10.48±0.24 (3.3%)
Total sugars	g	40.9±1.3	7.4±0.2
Fibre	g	3.9±0.5 (15.1%)	0.7±0.1 (2.7%)
Protein	g	9.6±0.3 (17.5%)	1.73±0.05 (3.2%)
Ashes	g	1.97±0.03	0.35±0.01
Sodium	mg	0.68±0.05 (0.3%)	0.12±0.01 (0.1%)

[a]The percentage of the daily recommended intake (DRI) of the nutrient which is satisfied by the ingestion of a 100 g portion of the snack or one snack (18 g) is indicated between parentheses. These values were determined considering a reference daily energy intake of 2000 kcal

On the other hand, the values predicted by the model for the response variables of F08 formulation and the values obtained experimentally were similar (Table 5) which was a good indicator of the reliability of the optimization method.

Finally, the optimal formulation presented a water activity that ensures the microbial stability (Tapia et al., 2007), a mesophilic count that indicates a satisfactory microbial quality (Gilbert et al., 2000) and sensory classification for Flavour and Texture above the minimum (5.0 points) defined for the sensorial acceptance of the product.

Nutritional composition of the optimized formulation
The optimized formulation was prepared with 50 g 100 g^{-1} of dehydrated sweet cherry, 35 g 100 g^1 of almond and 15 g 100 g^1 of honey, and baked for 13 min at 120ºC.

Table 6 shows the nutritional composition of this formulation. The snack has a high content of fat (16.8 g 100 g^1; 24.0% dietary reference intake, DRI), which came primarily from the almond; carbohydrates (58.2 g 100 g^{-1}; 18.2%DRI), essentially from the dehydrated sweet cherry and from the honey; protein (9.6 g 100 g^1; 17.5%DRI) and fibre (3.9 g 100 g^{-1}; 15.1%DRI), both mainly from the almond. Additionally, the saturated fatty acids content (1.5 g 100 g^{-1}) represents only 9% of the total fat content (16.8 g 100 g^{-1}).

According to the EC Regulation nº 1924/2006 (EC, 2006), the label of this snack could exhibit the following nutritional claims: "low saturated fat"; "with no added sugar"; "contains naturally occurring sugars"; "sodium free" or "salt free" and "source of fibre".

CONCLUSIONS

A new snack-bar formulation, based exclusively on sweet cherry, almond and honey, was developed and optimized through Response Surface Methodology and desirability function maximization. The optimal formulation, with 50 g 100 g^{-1} of sweet cherry, 35 g 100 g^{-1} of almond, 15 g 100 g^1 of honey and baked at 120ºC for 13 min, showed microbial stability due to its low water activity; a satisfactory microbial quality and a sensorial consumer acceptance. Furthermore, the nutrient analysis showed that the following claims could be printed in the label of this product: "low saturated fat"; "with no added sugar"; "contains naturally occurring sugars"; "sodium free" or "salt free" and "source of fibre".

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Authors' contributions
Cristina Miguel Pintado: conceptualization, methodology, supervision, writing – review and editing; Abel Veloso: data collection and analysis, writing – original draft; Zamira Maria: data collection and analysis, writing – original draft; Ana Silveira: laboratory analysis, data collection; Helena Beato: laboratory analysis, data collection; Luís Pinto de Andrade: supervision, writing – review and editing; Fernanda Delgado: conceptualization, supervision, methodology, writing – review and editing.

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