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Soy protein hydrolysate and gelling polysaccharides systems in cooked foams: a method to improve their combinations

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Abstract

One of the most important features of food systems is their multicomponent nature. Due to their interest in food industry mixed protein-polysaccharide systems are being increasingly studied. These systems are usually used to control structure, texture and stability of foodstuffs. In the particular case of this work, the focus is on mixtures of hydrolyzed soy protein (4% degree of hydrolysis), κ-carrageenan and hydroxypropylmethylcellulose.

The objective of this work was to study the proportion of each component of the mixed system to increase the times required to start the drainage and collapse without foam capacity decrease at heating food conditions. A statistic method was used as support to analyze these relations and estimate other possible combinations.

It was concluded that soy protein hydrolysate plays a mean role and should be at high concentrations in the mixed system, whereas, κ-carrageenan behaves better at lower concentration to obtain cooked foams with the desire properties.

Keyword: Foam, Soy protein, Hydrolysates, Polysaccharides, Response surface methodology

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Introduction

The main significance of the present work relies on the innovative way to know how combine habitual and inexpensive components in the food industry to improve functional properties by simple cooking conditions simulations.

Structural modifications allowing greater conformational flexibility of protein may improve their ability to stabilize foams and emulsions. Many studies have demonstrated that the enzymatic hydrolysis of soy proteins improves its functional properties, including solubility, emulsifying and foaming characteristics [1]. Nevertheless, it has been reported that limited hydrolysis may improve foaming capacity but decrease foam stability [2]. An adequate hydrolyzed soy protein-polysaccharides mixed system in an appropriate concentration would be useful to achieve acceptable foaming properties. Therefore, their use would require the addition of polysaccharides as stabilizers. Most high-molecular weight polysaccharides, being hydrophilic, do not have much of tendency to adsorb at the air-water interface, but they can strongly enhance the stability of protein foams by acting as thickening or gelling agents [2]. It was selected a hydroxypropylmethylcellulose that gels with temperature increase and κ-carrageenan that gels as temperature decrease.

In a previous work [3], we study the rheology, through elastic component (G´) and relative viscoelasticity (tan δ) and thermal transitions of continuous phase of same systems to determine their behavior at 70ºC. It was concluded that E4M (hydroxypropylmethylcellulose) was the principal component which determines the elastic and viscoelastic characteristics of complex mixture at 70ºC, due to the gelled structure produced at heating. In the basis of these results, we will intent to relation them with the foaming properties at 70ºC.

It was used soy protein hydrolysate, (HSP), hydroxyprophyl-methylcellulose (E4M) and a carrageenan (κC) with changes of concentration according to Doehlert matrix [4] used as experimental design. It was selected hot temperature (70 ± 5ºC) to simulate heating treatments, and study the behavior of the system foamed.

The response surface methodology was used to analyze the concentration effect of each component of the mixed system.

Materials and Methods

Materials

A commercial soy protein isolate (SP) (90% protein) from Sanbra, Brazil was used as substrate for the hydrolysis with fungal protease from *Aspergillus oryzae* with endopeptidase activity, provided by Quest International. The protein isolate was denatured as detected by differential scanning calorimetry. The following polysaccharides (PS), from Sanofi Bioindustries, Argentina, were used without further purification: κ-carrageenan (κC), and a hydroxypropylmethylcellulose (HPMC) called E4M from Dow Chemical Co.

Enzymatic hydrolysis

SP isolate (72 g in 1,200 ml of water) was hydrolyzed according to Zylberman et al., [5] batch-wise by treatment with fungal protease at pH 7.0, 50ºC for 1h, with enzyme/substrate (E/S) ratio: 2/100. Hydrolysis was stopped by heating at 80ºC for 10 min.

The variation in pH was very small (maximum decrease 0.3 pH units) and was adjusted back to the original value with diluted NaOH. Hydrolysates were lyophilized.

The degree of hydrolysis (DH), defined as the percentage of

peptide bonds cleaved, was calculated from the determination of free amino groups by reaction with *o-phthaldialdehyde* (OPA) according to Church et al. [6]. Protein hydrolysate with 4% DH (HSP) was obtained.

Foaming properties

Foam formation

20 ml of solutions were foamed at 60ºC where κC was not gelled [7], in a graduated tube (3 cm diameter) for 3 min with Griffin & George stirrer at 2500 rpm. Overrun was calculated as:

FO (%) = [(foam volume - 20) / 20] 100

The data reported are means of at least two replicates. The error was less than 10%.

Foam stability

After foaming production, the stability of foams simulating cooking process was studied by increasing the temperature at 70ºC. It was recorded the stability against liquid drainage and collapse of height of foams at these conditions.

It was evaluated the liquid drainage as the time to start the foam drainage (*lag time to drainage*, min). The foam collapse was calculated as the time required to start the collapse (*lag time to collapse*, min). The data reported are means of at least two replicates. The relative error for every measure was less than 10%.

Experimental design

The combined effect of a hydrolyzed soy protein (4% DH), a hydroxyprpylmethylcellulose called E4M and κ-carrageenan concentrations on foaming properties was evaluated by response surface methodology. A Dohelert design was selected to elaborate the experiment, which is associated with second order models detecting optimum values and interactions. This experiment involved 3 factors as independent variables where: x_1 = HSP; x_2 = E4M; x_3 = κC with 7, 5 and 3 levels of concentrations respectively. The concentrations used were 2 to10% wt/wt for HSP; 0.2 to 1.8% wt/wt for E4M and 0.2 to 1.8% wt/wt for κC.

The responses or dependent variables evaluated were the foam overrun at 60°C (*FO*); the foaming stability properties at heating conditions: lag time for start the foam drainage (*lag time to drainage*) and lag time for start the foam collapse (*lag time to collapse*)

A second-degree polynomial model was fitted for each dependent variable, as follows:

$$Y = bo + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2^2 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3$$

Where Y is the corresponding dependent variable, *bo*, *bii* and *bij* are regression coefficients and xi the coded independent variables, linearly related to HSP, E4M and κC levels. The Experimental matrix: real and coded (in brackets) values for the studied variables are shown in Table 1.

Statistical analysis

The model goodness-of-fit was evaluated by the coefficient of determination (R^2) and the analysis of variance (ANOVA). The response surface and contour plots were developed using the polynomial equations, obtained by holding one of the independent variables at a constant value and changing the levels of the other two variables using Statgraphics Plus 3.0. software.

Results and Discussion

Foaming properties

Foaming Overrun

Table 2 shows the results corresponding to foaming properties obtained for all combinations studied at heating (70ºC). It can be seen that *FO* value was inversely related with κC concentration in all EP (see also Table 1). κC addition limited the air incorporation to the foams due to the high viscosity imparted during the foam formation. It has been reported a similar behavior for foams of soy proteins in the presence of xanthan gum [8]. In same way Carp et al. [9], have been found similar results by using this polysaccharide on foams made from κ-lactoglobulin, native and denatured soy protein. It was shown that κC addition decreased foam expansion, because air incorporation was limited by increasing the viscosity of the solutions.

Table1: Experimental matrix: real and coded (in brackets) values for the studied variables.

EP	HSP(wt/wt)	E4M(wt/wt)	κC(wt/wt)
1	6 (0)	1 (0)	1 (0)
2	6 (0)	1.8 (1)	1 (0)
3	6 (0)	0.2 (-1)	1 (0)
4	10 (0.866)	1. 4(0.5)	1 (0)
5	2 (-0.866)	0.6 (-0.5)	1 (0)
6	2 (-0.866)	1.4 (0.5)	1 (0)
7	10 (0.866)	0.6 (-0.5)	1 (0)
8	7.33 (0.283)	1.4 (0.5)	1.8 (0.8165)
9	4.67 (-0.283)	0.6 (-0.5)	0.2 (-0.8165)
10	4.67 (-0.283)	1.4 (0.5)	0.2 (-0.8165)
11	8.67 (0.567)	1 (0)	0.2 (-0.8165)
12	7.33 (0.283)	0.6 (-0.5)	1.8 (0.8165)
13	3.33 (-0.567)	1 (0)	1.8 (0.8165)
14	6 (0)	1 (0)	1 (0)
15	6 (0)	1 (0)	1 (0)

EP: Experimental point; HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

Multiple regression analysis has been done for foaming parameters. The regression coefficients obtained are showed in Table 3.

In most cases R^2 resulted with a good fit and the "lack of fit" resulted non-significant (*FO*) which means that the model could explain the 80% of observed responses. In other cases, "lack of fit" resulted significant (lag times) which means that the order of the regression was not secondary (the model may not have included all appropriate functions of independent variables or the experimental region may be too large for a quadratic model was used). However, when a large amount of data was included in the analysis, a model with significant lack of fit could still be used [10].

It was observed for *FO* a significant linear variable for κC. The negative regression coefficient points out that the κC addition to foam measured at 60ºC conduces to a FO decrease, conforming it could be seen in the results (Table 2). The largest value indicated that it was the most important variable influencing the *FO*.

It also observed a significant negative linear coefficient corresponding to HSP, which indicates as similar way, a decrease of *FO* when HSP was present.

As κC addition, HSP would promote and increase of continuous phase viscosity leading to FO decrease. Other authors demonstrated that a decrease in foam expansion at high concentrations is believed to correspond to reduced solubility of protein solutions [11].

Figure 1 shows the corresponding response surface plot with

Table2: Experimental points (EP) and the corresponding results obtained for foaming properties at heating conditions (70ºC)

EP	*FO(%)**	*lag time drain.(min)**	*lag time to collapse(min)**
1	150	14.0	17.5
2	55	8.0	63.5
3	55	11.5	27.5
4	90	94.0	58.5
5	140	9.0	57.5
6	150	1.3	41.8
7	60	16.7	11.5
8	70	52.0	10.8
9	250	8.5	100.0
10	260	15.5	111.0
11	200	13.0	146.5
12	85	20.6	115.2
13	150	15.0	26.5
14	120	15.5	15.5
15	110	16.0	17.0

*Mean ± SD % less of 10% (2-8% for each EP) for foam overrun and drainage stability parameters of two replicates or more.
FO (%): foam overrun
Lag time to drainage: time to start the foam drainage.
Lag time to collapse: time required to start the collapse

the coded values of concentrations on the axes for *FO*. The graphic indicates that the highest *FO* was obtained when HSP and κC were in low concentrations. The behavior points out the great significance of κC in the decrease of *FO* for any HSP concentration as was stated before. However, comparing with HSP alone (data not shown); at 2% (wt) of concentration, 200% of *FO* was obtained, which indicates that the components concentrations could be extent to higher values to obtain foams with a reasonable *FO* with these mixed systems.

Lag time for foam drainage

Concerning to *lag time for foam drainage*, it was observed a significant influence of all components at heating conditions. The positive regression coefficients for HSP, E4M and κC indicate that in increasing concentrations of these components, the response increases, and HSP presented a major influence.

It was also observed synergistic interaction effects between HSP×E4M and HSP×κC, stronger in the first case. The different effects on the interactions between proteins and polysaccharides have been studied [12]. Under these experimental conditions of pH and temperatures, thermodynamic incompatibility would be favored and the effect of increasing the effective polysaccharide concentration would be the main explanation for the apparently increased foam stability of the mixed system. E4M gel formation would promote more stable foams against drainage and HSP×κC would promote the stability by viscosity increase of the continuous phase.

Figure 2 shows the response surface for this parameter with the coded values of concentrations on the axes. This response was significantly influenced by HSP and κC simultaneously. It can be seen that the *lag time for foam drainage* was the highest when both were at maximum concentrations at same time.

In similar way, drainage velocity were also measured (data not shown), however, times to start the foam drainage (lag times) were always sufficient to system gel, becoming consequently in a more

Table3: Model coefficients estimated by multiple linear regression for foaming properties

	FO	*lag time to drainage*	*lag time to collapse*
Constants	125.278	13.177	13.415
Linear			
HSP	-51.658**	27.212*	13.297*
E4M	(5.997)	11.433*	-3.692*
κC	-81.669*	11.802*	-39.449*
Quadratic			
HSP^2	(6.233)	19.088*	12.016*
$E4M^2$	(-69.783)	(-2.718)	33.244*
$κC^2$	83.312*	9.666*	99.748*
Interactions			
(HSP)×(E4M)	(3.109)	53.219*	56.760*
(HSP)×(κC)	(9.192)	14.356*	-23.927*
(E4M)×(κC)	(-13.323)	(0.884)	-83.168*
R^2	0.964	0.852	0.839
Lack of fit	NS	*	*

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.
*Significant value at P < 0.05; ** significant value at P < 0.01; and ( ) non-significant value.
Reduced equations for foaming properties:
FO = 125.278 – 51.685 HSP – 81.669 κC + 83.312 $κC^2$
lag time to drainage = 13.177 + 27.212 HSP + 11.433 E4M + 11.802 κC + 19.088 HSP^2 + 9.666 $κC^2$ + 53.219 HSP × E4M + 14.356 HSP×κC
lag time to collapse = 13.415+13.297 HSP - 3.692 E4M - 39.449 κC +12.016 HSP^2 + 33.244 $E4M^2$ + 99.748 $κC^2$ + 56.760 HSP×E4M -23.927 HSP×κC -83.168 E4M×κC

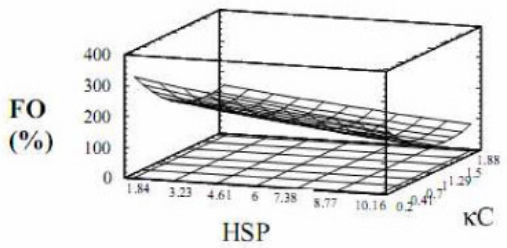

Figure 1: Foam overrun (FO) of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt).

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

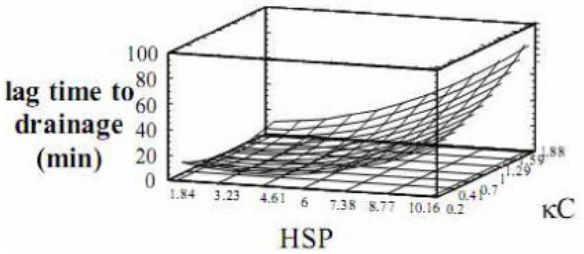

Figure 2: Lag time to drainage of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt).

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

appropriate drainage index of foams at 70ºC. That conclusion was made on the basis that 80 minutes for a *lag time for foam drainage* may be enough to heat the mixed system without the drainage takes place.

Lag time for foam collapse

In relation to the *lag time for foam collapse*, HSP showed a positive linear effect, increasing it by HSP addition, whereas the presence of E4M and κC decreased stronger this response. The detrimental influence of κC, would be related with the gelling capacity of E4M which can be impeded at κC high concentrations, leading to collapse of foams.

It can be also observed a synergistic interaction between HSP×E4M which induces a *lag time for foam collapse* increase at heating. At same time, HSP×κC and E4M×κC resulted as antagonist interactions by decreasing this response. Moreover, it can be observed that κC may provoke an increment of *lag time to collapse*. This indicates an unfavorable effect of κC on this parameter in it molten state at 70ºC, whereas, HSP, tended to favor the *lag time to collapse* by increasing it strongly. These results confirm that the types of interactions at heating conditions that conduce to drainage or collapse process are very different [13]. Evidently, molten κC presence would not favor the E4M gelation, which may be essential for collapse process of foams.

E4M (with the lowest significant coefficient) was kept constant at the central point value to generate the responses surfaces and contour lines plots for all responses studied. Figure 3 shows this response with the coded values of concentrations on the axes. It can be seen that principally HSP increased this response and κC decreased it. The response strongly decreases when κC is in increasing concentration value and it intensifies at high HSP concentrations as could be seen through the coefficients.

Since the l*ag time for foam collapse* of the HSP alone (data not shown) at 2% (wt) of concentration, was 1.3 minutes, and it can be said that the polysaccharides addition would always produce stabilization against the collapse start, which is very important for systems that go through heating process.

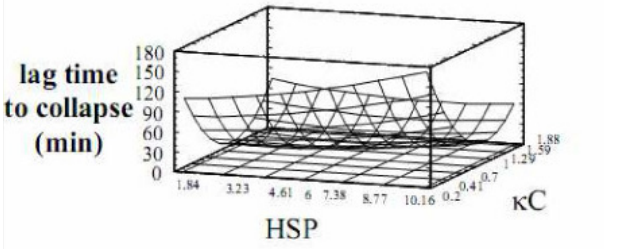

Figure 3: Lag time to collapse of foams corresponding to response surface plot as a function of concentration of HSP and κC in a concentration of E4M at central point replication (1%wt/wt).

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

The best combinations

To corroborate the exposed analysis, it was made the contour line plots keeping HSP concentration at high concentration (8.67% (wt)). Figure 4 a-c shows these plots with the coded values of concentrations on the axes for *FO*, *lag time for drainage* and *lag time for collapse* of foams at heating conditions respectively.

It can be seen that at high HSP concentration, high levels of κC produce an increase of the *lag time for foam drainage* (Figure 4b), but a same time produces a decrease of the *lag time for foam collapse* (Figure 4c) and *FO* (Fig. 4a). Hence, it can be concluded that this polysaccharide should be used in a low range of concentrations, such as 0.2 and 0.5% (wt). In addition, E4M at high HSP concentration, did not influence the *FO* (Figure 4 a) but was very significant for lag times (Figure 4b and 4c). Therefore, E4M should be used in a high range of concentrations, such as 1.5 and 1.8% (wt).

An analogous analysis was made for low HSP concentration, by keeping it at 2% (wt). In the Figure 5 a-c are shown the contour lines plots for *FO*, *lag time for foam drainage* and the *lag time for foam collapse* at heating respectively.

It can be observed that the highest *FO* (Figure 5a) was obtained at low κC concentrations (<0.5% (wt)). Similar results were obtained at high HSP concentrations (Figure 4 a), however, in this case, the *FO* values were higher than that.

Regarding the lag times for foams drainage and collapse, the polysaccharides influence at low HSP concentration, presented a different behavior. It can be seen in Figures 5b and 5c that the highest responses values were obtained in a broad concentration range of polysaccharides. For the *lag time for foam drainage* (Figure 5b), κC did not influence the response, whereas, E4M were optimized between 0.2 and 0.5% (wt) of concentration.

For *lag time for foam collapse* (Figure 5c) κC and E4M showed a huge influence, optimizing the response at low E4M concentrations (<0.5% wt) and high κC concentrations (> 0.5% wt) at heating

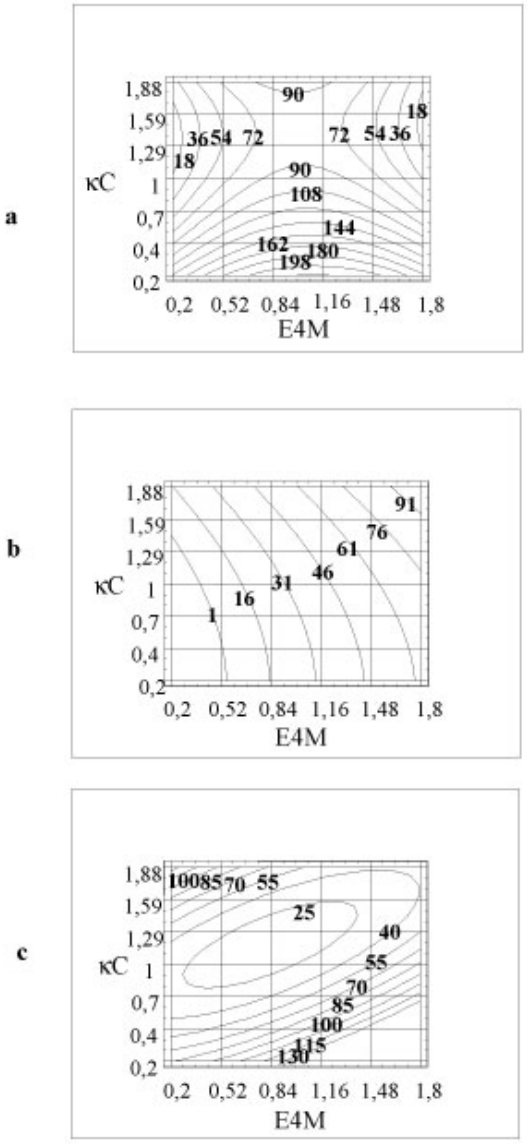

Figure 4:Contour lines plots at constant HSP = 8.67% (wt) for (a) FO, (b) lag time to drainage, and (c) lag time to collapse.

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

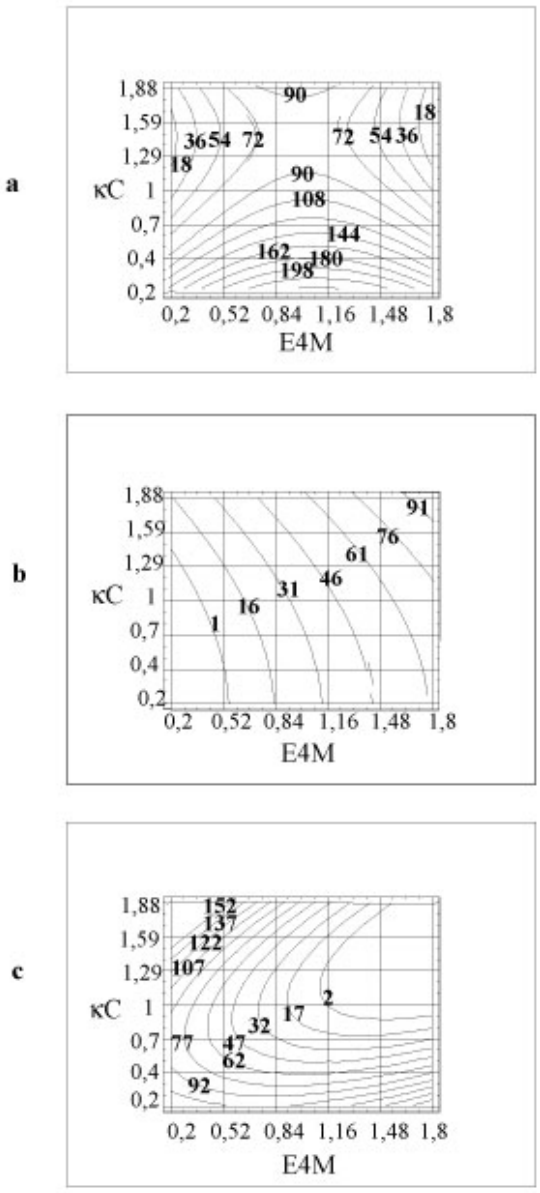

Figure 5:Contour lines plots at constant HSP = 2% (wt) for (a) FO, (b) lag time to drainage and (c) lag time to collapse.

HSP: Hydrolized soy protein at 4% DH; E4M: hydroxypropylmethylcellulose; κC: kappa-carrageenan.

Conclusions

conditions.

The polysaccharides addition in a combined way with hydrolyzed soy proteins is an adequate strategy to generate foams with a considerable stability against the liquid drainage and collapse of the foam over heating conditions, keeping a good foam capacity.

The huge stability increase at high temperatures was ascribed to a combined effect between the gelling character of E4M at 70ºC and the interaction with κC, which provides viscosity to the system.

Thus, the present methodology allows to optimize the biopolymers concentrations with the objective of formulate foamed systems with a reasonable foamability and good stability parameters at heating process. It was concluded that soy protein hydrolysate plays a mean role and should be at high concentrations in the mixed system, whereas, κC behaves better at lower concentration to obtain cooked foams with the desire properties.

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Author Contributions

Pilosof A: Designed the experiment and analyzed the data.
Martinez K: Wrote the article, analyzed the results and designed the discussion.

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