Physicochemical and rheological characterisation of microalgae-enriched ketchups and their sensory acceptability

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Abstract

Ketchup is one of the most popular tomato sauces in the restaurant and catering sector. Ketchup provides accents of colour and flavouring, as well as a smell and texture that is familiar and comforting. When compared to traditional products, alternative recipes make use of new ingredients like microalgae and sweeteners, well-known because of their functional and sensory properties. In this study, ketchups, with and without sugar addition, along with microalgae biomasses *Arthrospira platensis* (*Spirulina*) and *Chlorella vulgaris,* and *Dunaliella salina* extract at different concentrations, were prepared and evaluated. Colour differences regarding to the control samples were used to select microalgae concentrations. Physicochemical, rheological, and sensory properties of selected samples were characterised. Adding microalgae resulted in darker samples with intensified green, blue, and yellow hues. The use of microalgae in the preparation of ketchup had an impact on the features of the prepared products, increasing the apparent viscosity and consistency, and showing a more structured system compared to control samples. Furthermore, the microalgae incorporation in ketchup recipes affected taste intensity and sweetness, influencing ketchup's acceptance.

Keywords: *Chlorella vulgaris*, *Arthrospira platensis*, *Dunaliella salina* extract, ketchup, microalgae, acceptability.

1. Introduction

The tomato is one of the most important fruit products worldwide. It is the major dietary source of lycopene, flavonoids, and ascorbic acid (Fernández-García et al., 2012; Cárdenas-Castro et al., 2019); which have been linked to many health benefits, including a reduced risk of heart disease and cancer, due to their antioxidant and anti-inflammatory properties (Imran et al., 2020). Tomato is generally commercialised as a processed product (i.e. pastes, sauces, juices, and ketchup) (Patil Pandurang et al., 2020).

Ketchup is one of the most popular tomato products in the global market. It is a tomato-based sauce, which contains vinegar, sugar, salt, and various spices, used to modify or intensify the flavour and/or aroma of certain foods and culinary preparations. From the consumers' point of view, desirable characteristics of ketchup are red colour, high consistency, sweet and tomato taste (Ahouagi et al., 2021). However, the prevalence of overweight, obesity, and related non-communicable diseases remains high in all European countries (Blundell et al., 2017) making that diets must meet energy needs and provide a variety of foods of a high nutritional quality. This can be achieved by reducing or avoiding the sugar content of processed foods through reformulation (World Health Organization, 2020; Yusta-Boyo et al., 2020) and using novel ingredients such as microalgae biomass or their extract (Barkia et al., 2019).

The evolution of the food industry and gastronomy has been influenced by sustainability, affordability, accessibility, and cultural consumers acceptability (Guiné et al., 2020). Gastronomy has become gradually important, being recognised as artistic and cultural expression and fundamental pillars of family and social relationships (Cavicchi and Stancova, 2016). Furthermore, it offers the possibility to test new food developments suitable for industrial scaling up. Nevertheless, there is a great challenge to achieve consumer acceptance when products are not part of the consumer's culinary tradition

(Losada-Lopez et al., 2021) since using alternative ingredients in ketchup production can cause interesting modifications in quality as well as nutritional and sensory characteristics.

Different algae have entered the international cuisine because of their nutritional value and have found many applications. In western countries their consumption is on the rise (Mouritsen et al., 2018; Wendin and Undeland, 2020). For instance, recognised chefs like Ángel León pioneered using *Tetraselmis chuii* and *Nannochloropsis gaditana* in haute cuisine in Spain. However, microalgae is still scarcely considered in cuisine (Pérez-Lloréns, 2020), although they have been used for developing soups and snacks (Lafarga et al., 2019; Uribe-Wandurraga et al., 2019).

Microalgae have been used historically as a foodstuff, even as a delicacy or ancestral food in some cultures (Gouveia et al., 2008). The cyanobacterium *Arthrospira platensis* (*Spirulina*) also considered as microalgae, has a high protein content (65% of dry weight) and is a rich source of vitamins, especially vitamin B12 and provitamin A, minerals, especially iron, and is a natural source of γ-linolenic acid (Borowitzka, 2018). Likewise, *Chlorella vulgaris* is considered a potential source of a wide spectrum of bio-compounds (e.g., protein, fatty acids, carotenoids, vitamins, and minerals). In turn, *Dunaliella salina* extract has the highest content of β-carotene. Numerous benefits have been claimed for all of the mentioned nutritional compounds such as anti-inflammatory effects and health-promoting factors in many kinds of human body disorders (e.g. gastric ulcers, wounds, constipation, anaemia, hypertension, and diabetes) (Buono et al., 2014).

However, the addition of microalgal biomass to produce ketchup has not been thoroughly investigated so far. Despite its nutritional value, most consumers in Western countries are unfamiliar with seaweed and microalgae gastronomy and food production, which affect consumer attitudes and purchase intentions (Losada-Lopez et al., 2021).

Therefore, the combination of gastronomy with food industry will improve the knowledge and the satisfaction when tasting new products, developing new formulations by adding novel ingredients like microalgae and also, reducing or replacing sugar content.

The objective of this study was to investigate the effect of the addition of two kind of microalgae biomass, *Arthrospira platensis* and *Chlorella vulgaris;* and *Dunaliella salina* extract on pH, °Brix, colour, rheological characteristics, and sensory properties of sugar and with no added sugar ketchup formulations. The acceptability of microalgae-enriched ketchups by consumers was also evaluated, as microalgae have a distinct colour and taste history in the market, which may be useful for their commercialisation for home consumption and/or in the restaurants.

2. Materials and methods

2.1. Materials

Arthrospira platensis (*Spirulina*) and *Chlorella vulgaris* (*Chlorella*) freeze-dried biomasses were supplied by AlgaEnergy S.A. (Madrid, Spain). *Dunaliella salina* (*Dunaliella*) extract, by ROHA Europe S.L.U. (Torrent, Spain). Pulp tomato (28–30 °Brix), granulated sugar, modified starch, sweeteners mix, ketchup flavoured condiment, alcohol vinegar 10°, citric acid, and potassium sorbate were supplied by Jumel Alimentaria S.A. (L'Alqueria de la Comtessa, Valencia, Spain).

2.2. Preparation of the samples

The different ketchup samples (with or without sugar) were prepared according to the procedure provided by Jumel Alimentaria S.A. (L'Alqueria de la Comtessa, Valencia, Spain). Table 1 describes the ingredients and quantities used in the control recipe.

To prepare samples of ketchup with sugar addition (K), first, all ingredients were weighed separately (Table 1), and potassium sorbate was dissolved in 10 mL of water, due to its lower solubility in acidic medium (Ahouagi et al., 2021). Subsequently, water, pulp tomato, granulated sugar, modified starch, ketchup flavoured condiment, and the dissolved potassium sorbate were mixed in a kitchen appliance (Thermomix, TM31, Vorwerk Corporate Group, Wuppertal, Germany) at speed two (200 rpm) for 3 min. Afterwards, the mixture was heated to 75 °C until obtaining a homogeneous product. Lastly, alcohol vinegar and citric acid were added to the mixture and blended at speed two (200 rpm) until reaching 93 °C, then was blended for 1 min. The product was hot packaged in 500 mL polyethylene terephthalate (PET) bottles, cooled to 20 °C and stored at 4 °C for further studies.

To prepare samples of ketchup without sugar addition (SK), the same equipment and conditions were used as in the case of samples with added sugar. However, a sweetener mix, instead of granulated sugar, was added into the different formulations (Table 1). Samples with microalgae were developed using the same procedure but replacing water with different microalgae percentages (Table 2).

2.3. Experimental design

Minitab 18 Statistical Software (Minitab Inc., State College, PA, USA) was used for the surface response experimental design. A central composite design with three factors (microalgae content) and one replicate was obtained (Table 2). Upper and lower levels for each variable (microalgae) were determined according to previous sensorial trials (data not shown). The lower/upper levels used were 0.000/0.250% for *Spirulina*, 0.000/0.250% for *Chlorella*, and 0.000/0.120% for *Dunaliella*. The design was duplicated for both formulations, K and SK. Total colour differences (ΔE_1) between control samples (without microalgae) and the microalgae biomasses-extract, were used as the response variable.

2.4. Colour measurement

In order to determine the colour of the ketchup formulations, a Konica Minolta CM-700d colorimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with the standard illuminant D65 and visual angle of 10° was used to measure the CIEL*a*b* coordinates and subsequently, Chroma (C^*_{ab}), hue angle (h°_{ab}) and total colour differences (ΔE) of the samples were calculated according to Uribe-Wandurraga et al. (2021). Where ΔE_1 are the total colour differences between control samples and microalgae biomasses-extract samples for each type of formulations and ΔE_2, the total colour differences at the same level of microalgae biomasses-extract between K and SK. Samples were analysed by triplicate.

2.5. Physicochemical analysis

pH values of samples were measured using a pH-meter Crison MultiMeter MM41 (Hach Lange Spain, S.L.U., L'Hospitalet de Llobregat, Barcelona, Spain). Soluble solid content (SSC) of samples were measured with a digital pocket refractometer PAL-1 (ATAGO CO., LTD, Tokyo, Japan) and the results are reported as °Brix. Samples were analysed by triplicate at 20 °C on the same day of production.

2.6. Rheological and viscoelastic properties

Flow and oscillatory tests were performed using a Kinexus pro+ rotational rheometer (Malvern Instruments, Worcestershire, UK) and rSpace software (Malvern Instruments, Worcestershire, UK); equipped with a 25 mm diameter parallel-plate geometry (DSR II, Upper Plate) with a 1.0 mm gap between plates and a heat-controlled sample stage (Peltier Cylinder Cartridge, Malvern Instruments, Worcestershire, UK). Before each measurement, samples were loaded onto the geometry plate and rested for 5 min.

Flow tests were used to study the behaviour of shear stress on applied shear rate and viscosity profiles of samples. For better evaluation of flow behaviour of ketchups, a thixotropic loop measurement was conducted by first increasing the shear rate ($\dot{\gamma}$) logarithmically from 0.1 to 100 s^{-1} for 120 s and then, decreasing it logarithmically back to 0.1 for 120 s at 20 °C. For each up and down cycle, the area under the curve was calculated using SigmaPlot Software, version 11.0 (Systat Software Inc., San Jose, CA, USA). The difference between the two areas was the hysteresis loop area (Pa/s) (Torbica et al., 2016). The experimental data obtained for the first up sweep was well fitted to the power-law model (also known as the Ostwald-de Waele model) (equation 1),

$$\tau = k \cdot \dot{\gamma}^n \quad (1)$$

where τ is the shear stress (Pa), k is the flow consistency index ($Pa \cdot s^n$), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the flow behaviour index. Apparent viscosity (Pa·s) was recorded from the flow curve as a mean value at 100 s^{-1}. The parameters were calculated using SigmaPlot Software, version 11.0 (Systat Software Inc.).

The linear viscoelastic range for all the samples was determined using a strain sweep (0.001–100%) at a fixed frequency of 1.0 Hz, followed by oscillatory stress sweeps with a frequency range of 0.1–10 Hz for each sample using a constant strain of 0.01%. The mechanical spectra were obtained recording the elastic modulus (G' (Pa)) related to the material response as a solid and viscous modulus (G'' (Pa)) related to the material response as a fluid, both as a function of the frequency range (Hz). The loss angle values (tan) as a function of frequency (Hz) were calculated. Tan is defined as the ratio of G'' to G'.

For both tests, samples were performed on the day after preparations at 20 °C. Samples were analysed by triplicate.

2.7. Sensorial analysis

An untrained panel of 50 members, 23 men and 27 women, conducted a sensory analysis of the ketchup samples. The age of the panellists ranged from 28 to 55 years. The attributes evaluated were colour, colour intensity, taste, taste intensity, sweetness, sweetness intensity, consistency, and acceptability using a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) (AENOR, 2006).

During the test session, panellists worked in individual booths. All samples were presented to the panellists at 20 °C under normal lighting conditions in 30 mL cups with a 3-digit random number placed on them, identifying each sample. Panellists were given spoons to try the samples and room-temperature water to cleanse the palate before trying the next. At the session, the panellists evaluated eight ketchup samples, four for microalgae-enriched sugar addition and four, for microalgae-enriched with no added sugar.

2.8. Statistical analysis

Analysis of variance (ANOVA) using Statgraphics Centurion XVII Software, version 17.2.04 (Statgraphics Technologies, Inc. The Plains, VA, USA) with a confidence level of 95% ($p < 0.05$) was applied to evaluate the differences among physicochemical and rheological parameters, and ketchup-like attributes. The significance of differences between samples for each sensorial attribute was determined by applying Tukey's honestly significance difference (HSD) as a multiple comparison procedure (Meilgaard et al., 2006). Correspondence analysis was applied to the sensorial results using SPSS modeller 16.0 (IBM. New York, NY, USA).

3. Results and Discussion

3.1. Formulation and selection of ketchups

The formulation of the ketchups was developed based on company experience in creating formulations for the fruit processing industry, sugar ketchup (K) and with no added sugar ketchup (SK) (Table 1). To prepare the different microalgae-enriched mixtures, colour was the key parameter used to determine the amounts of each type of microalgae-extract into the different mixtures since colour is an important quality attribute of foods, which often determines consumer preference and acceptability (Intelmann et al., 2005). Thus, to determine the concentrations of a microalgae mix, a surface response experimental design was used considering the weight percent ranges of 0.000/0.250% for *Spirulina*, 0.000/0.250% for *Chlorella*, and 0.000/0.120% for *Dunaliella* extract (Table 2). These values were established from previous company studies. As a result, all the samples showed colour differences (ΔE_1) > 3 (Table 2), indicating a colour difference perceptible by the human eye (Bodart et al., 2008), but accepted by the company.

Figure 1 shows the response surface for ΔE_1 for *Spirulina* and *Dunaliella* extract. Intermediate *Spirulina* concentrations caused the largest differences in colour whereas low values produce the lowest variations. This is due to higher content of Chlorophyll a and Phycocyanin, naturally presented in *Spirulina*, resulting in darken samples (Buono et al., 2014; Igual et al., 2021). Uribe-Wandurraga et al. (2020, 2019) also observed this behaviour in microalgae-enriched emulsions and doughs. For *Dunaliella* and *Chlorella*, intermediate or low concentrations are required to reduce colour differences. In addition of the response surface methodology results, the values of ΔE_1 for these samples ranged between 7.4 and 9.2 for K samples and, between 4.9 and 6.9 for SK samples, compared to each control sample, CK and CSK, respectively (Table 2). Thus, K2, K3, K15, and K16 for microalgae-enriched sugar ketchups and, SK2, SK3, SK15, and SK16 for microalgae-enriched with no added sugar, were the samples chosen for further analyses, as they showed less significant ($p > 0.05$) colour differences (Table 2).

3.2. Physicochemical properties

Ketchup samples are preserved by low pH (adjusted by citric acid) and preservatives such as potassium sorbate, ingredients added during manufacturing processes. pH is the main quality parameter used to produce safe ketchups for consumers because of the presence of citric acid in formulations, which has significant influence on the microbiological stability of the final products (Lücke, 2003; Patil Pandurang et al., 2020). pH values of all the tested ketchups ranged between 3.62 and 3.70 (Table 3) agreeing with The Commission of the European Communities, (1986) regulation for tomato-based products.

Soluble solid content (SSC) is an important factor because of the higher the total solids the better the quality of the end product will be (Sharoba et al., 2005). No significant ($p > 0.05$) differences were observed for the SSC between K samples, which ranged from 31.80 to 31.97 (°Brix), whereas there were slight significant ($p < 0.05$) differences, between SK samples, ranging from 30 to 31 (°Brix) (Table 3). Thus, pH and the SSC of the ketchup formulations of this study are in accordance with commercial ketchup brands (Sharoba et al., 2005).

3.3. Colour

Colour of the product can also be used as an indicator of freshness because extensive processing and the use of different ingredients can often lead to the deterioration of the typical colour (Intelmann et al., 2005). Colour parameters, lightness (L*), redness (a*), yellowness (b*), hue (h^{o}_{ab}), and chroma (C^*_{ab}), decreased significantly ($p < 0.05$) due to microalgae biomasses/extract mix addition for both, K and SK samples, at different concentrations, compared to each control sample (Table 3). Adding microalgae biomasses/extract in samples, resulted in changes of the samples colour, causes them to darken, K15 (L* = 9.8) and SK2 (L* = 12.2), in relation to each control ketchup. When *Spirulina*, *Chlorella*, and *Dunaliella* extract are added at 0.125, 0.000, and 0.060 %, respectively, for SK15 and at 0.000, 0.125, and 0.060 %, respectively, for K16 and SK16 samples, low values of a*, b*, and C^*_{ab} were observed, meaning green, blue, and yellow hues are intensified. These changes of the colour parameters may be affected by the phycocyanin pigment in *Spirulina* (Park et al., 2018), Chlorophyll a for both *Spirulina* and *Chlorella* (Igual et al., 2021), lutein pigment in *Chlorella* (Dufossé, 2016) and β-carotene pigment in *Dunaliella* (Buono et al., 2014). These results may also suggest that the reaction kinetics of pigment degradation, upon high temperature processing, might depend on the initial pigment concentration (Batista et al., 2019). Nevertheless, the hue angle remained relatively constant for all the microalgae biomasses/extract mix samples, independent of the concentration. Therefore, the samples colour was deeply related to the microalgae's pigment profile whereas the heating procedure might have affected its intensity or chromaticity. Comparing K and SK samples to CK and CSK, respectively, all the samples showed $\Delta E_1 > 3$, detectable by the human eye (Bodart et al., 2008). However, K3, K15, SK2, and SK3 showed fewer colour differences, meaning that they could be more attractive to consumers. With K samples, higher colour differences regarding the control sample, could be produced by Maillard browning reaction and/or caramelisation due to sugar content. Because they are fundamental to the formation of colour in several food products (Goldfein and Slavin, 2015). Between K and SK samples, K2 compared to SK2 and K15 compared to SK15 showed $\Delta E_2 < 3$, meaning not perceptible to the human eye (Bodart et al., 2008) (Table 3).

3.4. Rheological and viscoelastic characterisation

Rheological properties of ketchups were investigated by measuring their flow behaviour and dynamic rheological properties, important in the design of texture, and in storage and processing stability measurements (Sahin and Ozdemir, 2004).

The viscosity of samples was evaluated using flow sweep tests and presented as the viscosity (η) as a function of the shear rate ($\dot{\gamma}$). Figures 2a and 2b show results for K and SK, respectively. In all cases, viscosity decreases as shear rate increases, giving shear-thinning behaviour (Koocheki et al., 2009). The viscosity of ketchups is enhanced due to the pectin substances naturally presents in fruits (Sahin and Ozdemir, 2004).

To evaluate the flow behaviour of the samples, the flow curves, plotting the experimental shear stress (Pa) as a function of the shear rate (s^{-1}) data, are shown in Figures 2c and 2d. To quantitatively compare ketchups, the experimental data were fit to the Ostwald-de Waele or power-law model. Proper fitting of the model was confirmed by R^2 values varying from 0.995 to 0.999 (Table 4). The behaviour of all the ketchup samples confirms a non-Newtonian plastic and dependent shear-thinning (thixotropic) behaviour between the shear stress and the shear rate over the whole studied range (Figures 2c and 2d). These results agreed with other authors who also reported this behaviour in tomato-based products (Koocheki et al., 2009; Torbica et al., 2016).

Apparent viscosity, hysteresis area, the model obtained parameters, and the dynamic oscillatory test results of the different samples are shown in Table 4.

The apparent viscosity of the samples increased when increasing the quantity of the microalgae biomasses/extract mix, with a steep increase for samples SK2, SK16, K15 and K16. Such an increase indicates a possible strengthening effect of the sample structure, believed to be due to a reinforcement of the viscoelastic protein matrix as a result of the addition of microalgae with high protein content (Uribe-Wandurraga et al., 2020). These results agree with Nova et al. (2020), who reported that microalgae addition improved apparent viscosity of food products. The increase of the apparent viscosity may also be influenced by the total solid content of the ketchups, increasing during processing as a function of sample dehydration/concentration processes (Torbica et al., 2016). Furthermore, enzymatic degradations and pectin/protein matrix interaction may also affect the consistency of tomato-based products (Sahin and Ozdemir, 2004).

The hysteresis area provides an insight into the sample network structure where a greater loop area suggests more structured fluids and vice versa (Chhabra, 2010). Here, K15 and SK2 had a comparatively more structured system compared to CK and CSK, respectively, showing significant differences ($p < 0.05$).

The consistence coefficient (k) of the samples (Table 4) showed a significant difference ($p < 0.05$) in K15 and K16 values compared to CK, and SK2 value compared to CSK. Higher k values in samples indicate a more pronounced viscous characteristic, which corresponds to a stronger network structure. Microalgal biomass can contribute to the reinforcement of the sample structure through the formation of physical entanglements (Raymundo et al., 2005). These results are consistent with the apparent viscosity and hysteresis loop of same samples (K15 and SK2). Therefore, the microalgae biomasses and extract addition in the ketchup structures was reinforced, observed as a higher resistance to structural breakdown (Uribe-Wandurraga et al., 2020).

The flow behaviour indicator (*n*) is an index to recognise the properties of the liquid product; $n < 1$, a shear-thinning liquid, $n = 1$, Newtonian fluid, and $n > 1$, a shear-thickening or dilatant fluid. No significant ($p > 0.05$) differences for SK samples, with *n* ranging from 0.25233 to 0.2600, and significant ($p < 0.05$) differences for K samples with *n* ranging from 0.2350 to 0.2740 with microalgae biomasses/extract addition compared to CSK and CK, respectively, were observed. Therefore, all samples show shear-thinning behaviour with a flow behaviour index $n < 1$ (Table 4), characteristic for ketchups. Similar values were obtained for other authors when adding porang flour in tomato ketchup (Mubarok and Ananda, 2020).

Figures 3a and 3b show the dynamic mechanical spectra of the ketchup samples as functions of frequency. Elastic modulus (G') was higher than the viscous modulus (G'') for all samples, and both parameters progressively increased throughout the studied frequency range. Moreover, loss angle (tan) values for all ketchup samples were larger than 0.1 (Table 4). This behaviour may be classified rheologically as a weak gel behaviour (Mansouripour et al., 2017) which agrees with previous reports on tomato-based products (Koocheki et al., 2009; Juszczak et al., 2013).

Compared to CK, K15 presented the highest G' and G'' values for this formulation, whereas for CSK, SK2 and SK3 presented the highest G' and G'' values showing no significant ($p > 0.05$) differences (Table 4). This can relate to *Spirulina* and *Chlorella* having a high protein content (Buono et al., 2014), giving a slight reinforcement of the structure (Batista et al., 2011).

3.5. Sensory properties

Figure 4 shows the sum of scores of each sample with sugar (a) and with no added sugar (b) for each evaluated attribute. Regarding K samples (Figure 4a), K2 showed the highest sensory colour intensity, followed by the K15 and K16, which is consistent with the C^*_{ab} sample values (Table 3). The ketchup with the lowest taste and taste intensity detected by the assessors was K16. Furthermore, K15 showed the highest sweetness intensity scores, whereas K3 and K16 the lowest. Assessors found no major differences in the ketchup's consistency. The ketchup samples are ordered according to their acceptability as K15 > K2 > K3 > K16. With SK samples (Figure 4b), assessors found differences in the ketchup's consistency, SK2 showed the highest consistency and SK15 the lowest. However, SK15 showed the highest taste intensity, even higher than ketchups with sugar added (Figure 4a). SK samples are ordered according to their acceptability as SK15 > SK3 > SK2 > SK16. In both cases, sample 15 (K15 and SK15) showed the highest acceptability by assessors. In contrast, K16 and SK16 showed the lowest acceptability by assessors. Therefore, K15 and SK15 were the best accepted samples, for both, sugar and with no added sugar ketchup formulations.

The HSD method applied to the sum of ranks was used to perform a multiple comparison among the treatments. The calculated Tukey's HSD value according to assay conditions was 31.53. When the difference between the sums of rank of each pair of samples, for each attribute, was greater than 31.53, significant differences between paired samples were assumed. Table 5 shows the differences between the sums of ranks for each pair of samples for the studied attributes. Colour, consistency, and acceptability showed no significant differences between paired samples. For K samples (Table 5a), the significant differences between pairs were K2-K16 in taste, K3-K15 in sweetness intensity, and K15-K16 in taste. The most similar for SK samples (Table 5b), were SK2 and SK16 and the most different were SK3 and SK15. Comparing K and SK formulations

(Table 5c), samples showed greater differences in attributes related to taste and sweetness. K16-SK16 showed no significant differences in any attribute. Therefore, in sensory terms, these samples were not affected by the adding of sugar or not.

To relate the different ketchups obtained with the attributes evaluated and the acceptability of the assessors, a correspondence analysis was conducted. From this analysis, all the ketchups attributes measured could be combined using two dimensions that explain 76% of the variability of results. The first dimension explained 51% of the variability, whereas the second explained 25%. Table 4 shows that both, the ketchups and the attributes, were well represented along the first two dimensions because high values were obtained for the sum of the relative contributions in all cases. Figure 5 shows the projection of the ketchups and attributes in the corresponding normalised plane; the values were calculated from Table 4. In this figure, the closer the obtained points, the greater the relation among them. According to the distribution of attributes and samples in the plane; SK2 was identified with higher consistency, K16 with colour intensity, and K15 with sweetness. The projections of SK2 and SK3 on D2 and D1, respectively, showed that they presented greater intensity of sweetness. These samples are associated by assessors with sweeter samples probably due to the sweetener´s higher sweetening capacity than that of common sugars. Moreover, samples K2, K3, SK2 and SK3 had lower values of *Chlorella* and *Spirulina* (0.096 % in total), meaning that the perception of salty and marine taste is lower compared to samples K15, K16, SK15 and SK16 with 0.125 % (Table 2). It is worth mentioning that *Dunaliella* extract does not affect the taste since it is used as a colouring agent. K3 was associated with a high score in colour, agreeing with its own colour parameters (Table 3) and taste intensity. Furthermore, it can be observed that taste intensity and sweetness were closely related to the acceptability of the assessors because they were near in representation. This shows that taste intensity and sweetness have considerable influence on ketchup's acceptance by the assessors. K2 and K3 showed more acceptability than the other samples.

4. Conclusions

Microalgae can be used as ingredient in sugar and with no added sugar ketchup formulations as functional ingredient. Aspects related with colour, viscosity or taste must be taken in account to guaranty acceptability of the product. The addition of microalgae increases the apparent viscosity and consistency and show a more structured system compared to control samples. Also, the quantity of microalgae added can affect significantly colour of samples, thus the equilibrium between quantity of microalgae and colour variations must be considered to avoid effect on acceptability. Furthermore, the microalgae incorporation in ketchup recipes affected taste intensity and sweetness, influencing ketchup’s acceptance. Therefore, it can be said that for both type of ketchup samples, sugar added and with no added sugar, formulation K15 and SK15, showed the highest acceptability by assessors as well as the best physicochemical properties.

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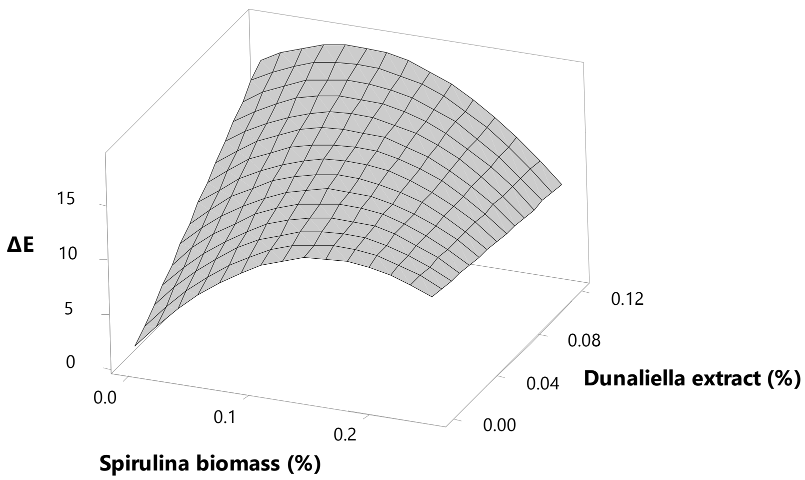
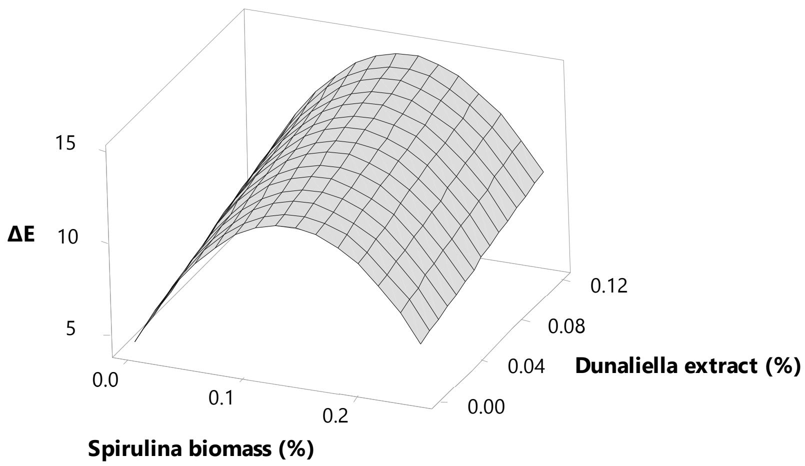

ΔE
15
10
5
0
0.0
0.1
0.2
Spirulina biomass (%)
0.12
0.08
0.04
0.00
Dunaliella extract (%)

a)


ΔE
15
10
5
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Spirulina biomass (%)
0.12
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0.04
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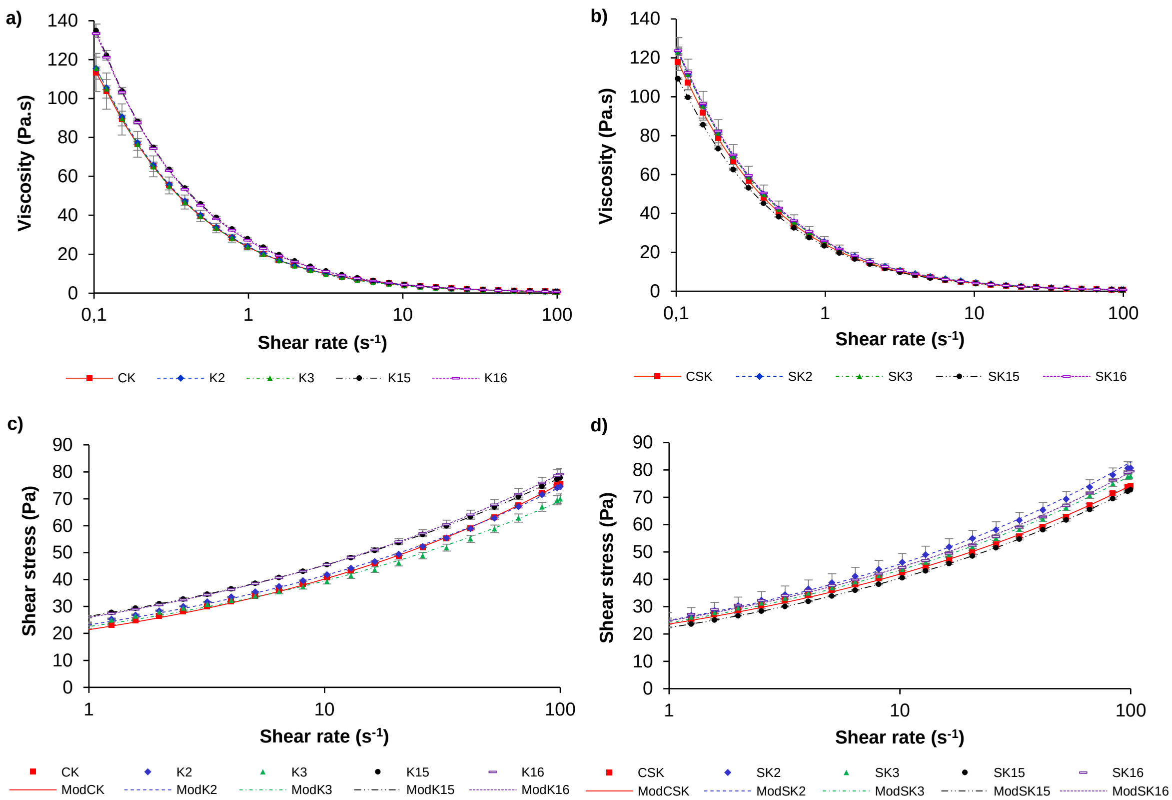
Dunaliella extract (%)

b)

Figure 1.



a)
Viscosity (Pa.s)
Shear rate (s-1)
CK K2 K3 K15 K16
b)
Viscosity (Pa.s)
Shear rate (s-1)
CSK SK2 SK3 SK15 SK16
c)
Shear stress (Pa)
Shear rate (s-1)
CK K2 K3 K15 K16
ModCK ModK2 ModK3 ModK15 ModK16
d)
Shear stress (Pa)
Shear rate (s-1)
CSK SK2 SK3 SK15 SK16
ModCSK ModSK2 ModSK3 ModSK15 ModSK16

Figure 2.

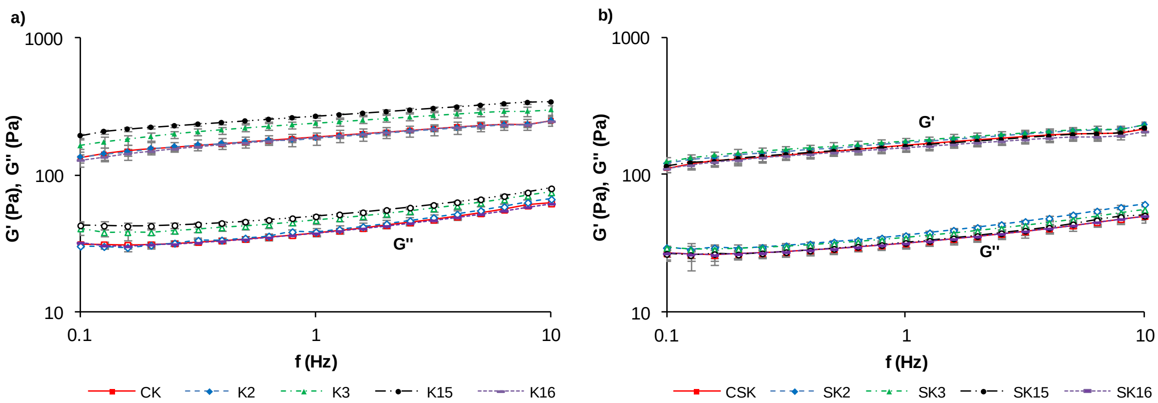

a)
1000
100
10
G' (Pa), G'' (Pa)
G''
0.1
1
10
f (Hz)
CK
K2
K3
K15
K16
b)
1000
100
10
G' (Pa), G'' (Pa)
G'
G''
0.1
1
10
f (Hz)
CSK
SK2
SK3
SK15
SK16

Figure 3.

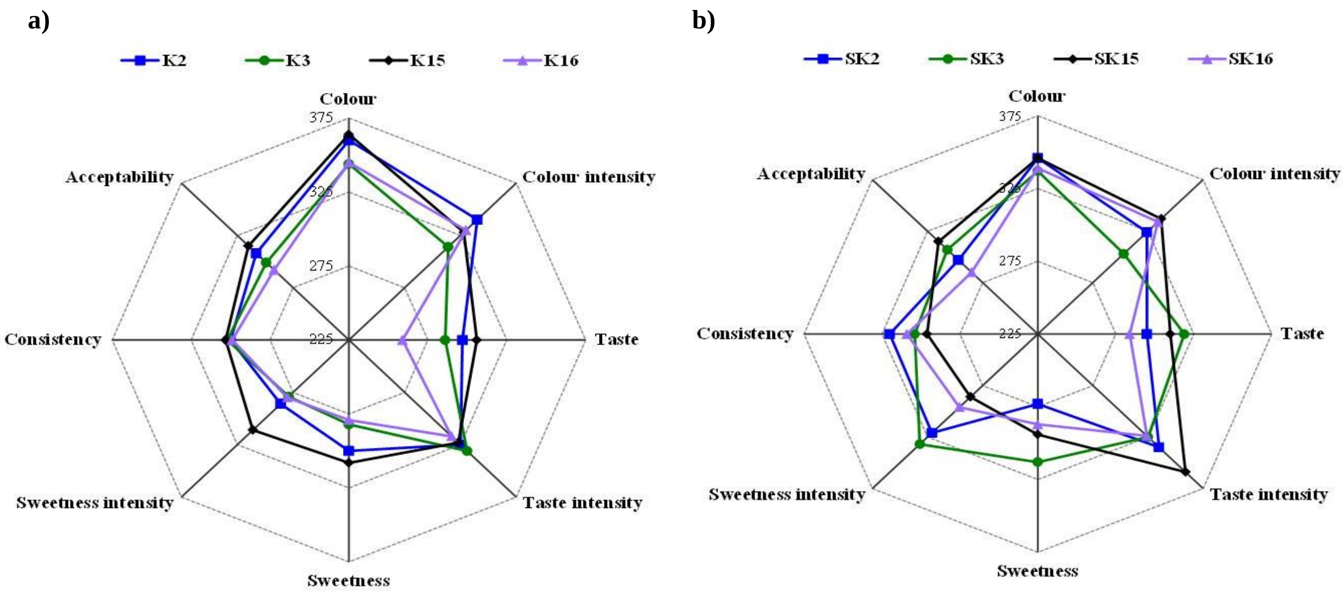

a)
K2
K3
K15
K16
Colour
375
325
275
225
Colour intensity
Taste
Taste intensity
Sweetness
Sweetness intensity
Consistency
Acceptability
b)
SK2
SK3
SK15
SK16
Colour
375
325
275
225
Colour intensity
Taste
Taste intensity
Sweetness
Sweetness intensity
Consistency
Acceptability

Figure 4.

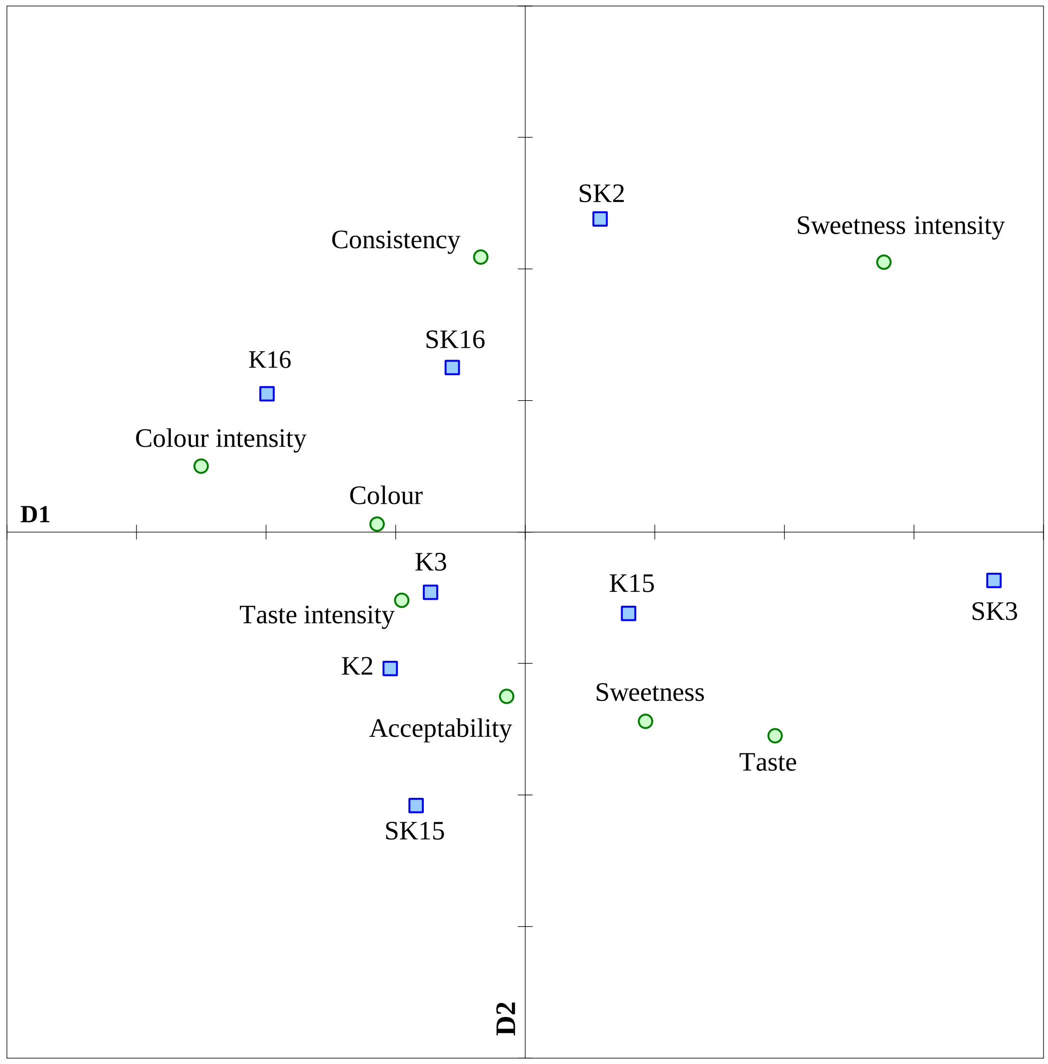

SK2
Sweetness intensity
Consistency
SK16
K16
Colour intensity
Colour
D1
K3
Taste intensity
K15
SK3
K2
Sweetness
Acceptability
Taste
SK15
D2

Figure 5.

Figure captions

Figure 1. Surface response for colour differences (ΔE_1) versus Spirulina biomass and Dunaliella extract concentrations of a) Ketchup (K) and b) No added sugar ketchup (SK).

Figure 2. Viscosity profiles of a) Ketchup (K) and b) No added sugar ketchup (SK) at different microalgae-mix incorporation levels and, Flow behaviour of the assayed ketchups. Values obtained from Ostwald-de-Waele model (continuous and dotted lines) and Shear stress (σ) vs Shear rate ($\dot{\gamma}$) (bookmarks) of c) Ketchup (K) and d) No added sugar ketchup (SK) at different microalgae-mix incorporation levels. C: Control; 2, 3, 15, and 16: Microalgae-mix-enriched samples.

Figure 3. Frequency sweeps of ketchups. Elastic modulus (G') and viscous modulus (G") of a) Ketchup (K) and b) No added sugar ketchup (SK) at different microalgae-mix incorporation levels. C: Control; 2, 3, 15, and 16: Microalgae-mix-enriched samples.

Figure 4. Score of the different sensory attributes evaluated in a) Ketchup (K) and b) No added sugar ketchup (SK). Concentric octagonal isolines show the axis tick marks.

Figure 5. Correspondence analysis. Representation of attributes and samples tested in the normalised plain defined by the two dimensions explaining the variability of the results of the sensorial analysis.

Table 1. Ingredients (%, w/w) used in the control ketchup formulations.

Ketchup (K)		No added sugar ketchup (SK)	
Ingredients	**%**	**Ingredients**	**%**
Water	44.49	Water	44.49
Tomato pulp (28-30 °Brix)	25.50	Tomato pulp (28-30 °Brix)	25.50
Granulated sugar	22.20	Modified starch	13.59
Alcohol vinegar 10%	3.80	Sweeteners mix	9.02
Ketchup flavoured condiment	2.15	Alcohol vinegar 10%	4.23
Modified starch	1.16	Ketchup flavoured condiment	2.47
Citric Acid	0.60	Citric Acid	0.60
Potassium sorbate	0.10	Potassium sorbate	0.10
Total	100	Total	100

Table 2. Mixing design of *Arthrospira platensis* (*Spirulina*) biomass, *Chlorella vulgaris* (*Chlorella*) biomass and *Dunaliella salina* (*Dunaliella*) extract and their corresponding amount of water and, colour differences (ΔE) for both, ketchup (K) and with no added sugar ketchup (SK) formulations.

Sample	*Spirulina* biomass (%)	*Chlorella* biomass (%)	*Dunaliella* extract (%)	Water (%)	ΔE_1K	ΔE_1SK
1	0.048	0.202	0.097	44.143	11.7 (0.3)^{c}	11.9 (0.7)^{d}
2	0.048	0.048	0.097	44.296	8.3 (1.6)^{ab}	4.9 (0.4)^{a}
3	0.048	0.048	0.023	44.370	7.4 (1.4)^{a}	5.69 (1.08)^{a}
4	0.202	0.202	0.023	44.064	18.8 (1.2)^{h}	17.6 (1.5)^{h}
5	0.125	0.125	0.060	44.180	14.4 (1.3)^{fg}	12.2 (1.2)^{de}
6	0.202	0.202	0.097	43.990	19.9 (1.6)^{i}	10.2 (0.5)^{c}
7	0.048	0.202	0.023	44.217	14.4 (1.2)^{fg}	13.8 (1.4)^{gh}
8	0.202	0.048	0.097	44.143	14.1 (1.2)^{efg}	12.3 (0.7)^{de}
9	0.125	0.125	0.060	44.180	14.1 (1.4)^{efg}	12.9 (0.9)^{efg}
10	0.125	0.125	0.060	44.180	13.4 (0.9)^{def}	12.0 (1.2)^{d}
11	0.202	0.048	0.023	44.217	11.4 (1.2)^{c}	13.2 (0.9)^{fg}
12	0.125	0.125	0.060	44.180	13.4 (1.2)^{def}	12.2 (1.4)^{de}
13	0.125	0.125	0.060	44.180	14.4 (0.9)^{fg}	12.2 (0.7)^{de}
14	0.125	0.125	0.060	44.180	13.3 (0.7)^{de}	12.7 (0.7)^{def}
15	0.125	0.000	0.060	44.305	7.4 (0.9)^{a}	6.9 (0.6)^{b}
16	0.000	0.125	0.060	44.305	9.2 (0.8)^{b}	6.6 (0.5)^{b}
17	0.125	0.125	0.120	44.120	12.9 (0.9)^{d}	12.5 (0.7)^{def}
18	0.125	0.250	0.060	44.055	19.2 (0.8)^{hi}	14.2 (0.9)^{g}
19	0.250	0.125	0.060	44.055	19.4 (0.9)^{hi}	17.7 (0.8)^{h}
20	0.125	0.125	0.000	44.240	15.0 (1.4)^{g}	12.5 (0.8)^{def}

The same letter in superscript within columns indicates homogeneous groups established by the ANOVA ($p < 0.05$). Colour differences of each type of microalgae-enriched ketchup and with no added sugar microalgae-enriched ketchup as compared to each control (ΔE_1).

Table 3. pH, Soluble solid content (SSC, °Brix) and colour parameters of ketchup samples

Samples		pH	°Brix	L*	a*	b*	h^{o}_{ab}	C^*_{ab}	ΔE_1	ΔE_2
Ketchup (K)	CK	3.68 (0.03)^{a}	31.83 (0.15)^{a}	12.5 (0.3)^{d}	33.9 (0.2)^{c}	21.6 (0.5)^{d}	32.5 (0.6)^{d}	40.2 (0.3)^{d}	-	-
	K2	3.670 (0.010)^{a}	31.80 (0.10)^{a}	9.2 (0.7)^{b}	29.2 (0.5)^{a}	15.8 (1.2)^{b}	28 (2)^{ab}	33.2 (0.8)^{b}	8.3 (1.6)^{ab}	-
	K3	3.68 (0.02)^{a}	31.80 (0.10)^{a}	9.5 (0.7)^{bc}	29.7 (0.2)^{b}	16.4 (1.2)^{bc}	29 (2)^{bc}	33.9 (0.6)^{c}	7.4 (1.4)^{a}	-
	K15	3.70 (0.02)^{a}	31.97 (0.12)^{a}	9.8 (0.6)^{c}	29.2 (0.6)^{a}	16.8 (1.1)^{c}	30 (2)^{c}	33.7 (0.5)^{c}	7.4 (0.9)^{a}	-
	K16	3.687 (0.012)^{a}	31.87 (0.15)^{a}	8.6 (0.5)^{a}	29.2 (0.3)^{a}	14.8 (0.9)^{a}	26.9 (1.3)^{a}	32.7 (0.5)^{a}	9.2 (0.8)^{b}	-
No added sugar ketchup (SK)	CSK	3.650 (0.010)^{bc}	30.73 (0.15)^{b}	13.6 (0.3)^{c}	34.1 (0.3)^{c}	23.4 (0.5)^{c}	34.5 (0.6)^{b}	41.4 (0.3)^{c}	-	2.1 (0.7)^{a}
	SK2	3.623 (0.012)^{a}	30.90 (0.10)^{b}	12.2 (0.3)^{b}	30.2 (0.5)^{ab}	21.0 (0.5)^{b}	34.77 (1.02)^{b}	36.8 (0.4)^{b}	4.9 (0.4)^{a}	6.1 (1.7)^{c}
	SK3	3.633 (0.012)^{ab}	30.93 (0.06)^{b}	11.4 (0.5)^{a}	30.6 (0.8)^{b}	19.6 (0.8)^{a}	32.69 (1.02)^{a}	36.33 (0.98)^{b}	5.69 (1.08)^{ab}	4.0 (1.9)^{b}
	SK15	3.653 (0.012)^{c}	31.0 (0.2)^{b}	11.0 (0.2)^{a}	29.5 (0.2)^{a}	19.0 (0.4)^{a}	32.7 (0.6)^{a}	35.1 (0.3)^{a}	6.9 (0.6)^{c}	2.69 (1.09)^{a}
	SK16	3.627 (0.006)^{a}	30.0 (0.2)^{a}	11.2 (0.4)^{a}	29.5 (0.4)^{a}	19.3 (0.6)^{a}	33.23 (1.09)^{a}	35.3 (0.3)^{a}	6.6 (0.5)^{bc}	5.3 (1.2)^{c}

The same letter in superscript within column for each type of formulation sample (Ketchup or No added sugar ketchup) indicates homogeneous groups established by ANOVA ($p < 0.05$). Colour differences of each type of microalgae-enriched ketchup and with no added sugar microalgae-enriched ketchup as compared to each control (ΔE_1) and, compared amongst ketchup and with no added sugar ketchup at the same level of microalgae-mix fortification (ΔE_2).

Table 4

Table 4. Apparent viscosity, hysteresis loop area (S), the Ostwald-de Waele model parameters (consistency index (k), flow behaviour index (n), and coefficient of correlation (R^2_{adj})), and dynamic oscillatory test results at 1 Hz (storage modulus (G'), loss modulus (G'), and tan δ) of studied ketchup samples.

Samples		Apparent Viscosity (Pa.s) at 100 s^{-1}	Ostwald–de Waele model $\tau = k \cdot \dot{\gamma}^n$ First up sweep				G' (Pa)	G'' (Pa)	tan δ
			Hysteresis loop area. S (Pa/s)	Consistency index. k (Pa.s^n)	Flow behaviour index. n	R^2adj			
Ketchup (K)	CK	0.743 (0.009)^b	480 (11)^c	21.387 (0.001)^a	0.274 (0.001)^d	0.999	188 (8)^a	38 (2)^a	0.199 (0.002)^{bc}
	K2	0.728 (0.005)^b	524 (41)^{cd}	23.1754 (0.0004)^b	0.2532 (0.0004)^c	0.998	187 (19)^a	38 (2)^a	0.205 (0.008)^c
	K3	0.694 (0.019)^a	313 (11)^a	22.587 (0.002)^b	0.243 (0.002)^b	0.997	239 (13)^b	47 (3)^b	0.195 (0.005)^b
	K15	0.811 (0.11)^c	553 (17)^d	26.358 (0.002)^c	0.235 (0.002)^a	0.995	268 (8)^c	49.6 (1.2)^b	0.185 (0.002)^a
	K16	0.804 (0.007)^c	408 (52)^b	25.992 (0.007)^c	0.242 (0.007)^b	0.997	188 (3)^a	37.3 (0.9)^a	0.199 (0.003)^{bc}
No added sugar ketchup (SK)	CSK	0.714 (0.002)^a	669 (46)^b	23.618 (0.003)^{ab}	0.249 (0.003)^a	0.998	163 (12)^{ab}	33 (2)^{ab}	0.2003 (0.0004)^{ab}
	SK2	0.781 (0.014)^c	757 (26)^c	25.04 (0.02)^b	0.26 (0.02)^a	0.998	171 (6)^{ab}	35.9 (1.2)^b	0.2103 (0.0012)^c
	SK3	0.756 (0.009)^b	639 (52)^b	24.106 (0.002)^{ab}	0.255 (0.002)^a	0.999	175 (10)^b	34 (2)^{ab}	0.197 (0.002)^a
	SK15	0.709 (0.002)^a	476 (50)^a	22.3805 (0.0003)^a	0.2559 (0.0004)^a	0.998	159 (12)^{ab}	32 (2)^a	0.199 (0.003)^{ab}
	SK16	0.785 (0.007)^c	415 (39)^a	24.8075 (0.0008)^b	0.2523 (0.0008)^a	0.998	156 (10)^a	31 (2)^a	0.201 (0.002)^b

The same letter in superscript within column for each type of formulation sample (Ketchup or No added sugar ketchup) indicates homogeneous groups established by ANOVA ($p < 0.05$).

Table 5. Differences between the sums of ranks for each pair of samples with sugar (a), with no added sugar (b), and comparing with-without sugar (c) for attributes

a)	**K2-K3**	**K2-K15**	**K2-K16**	**K3-K15**	**K3-K16**	**K15-K16**
Colour	16	4	15	20	1	19
Colour intensity	26	12	10	14	16	2
Taste	11	9	38*	20	27	47*
Taste intensity	14	2	8	8	14	6
Sweetness	18	8	21	26	3	29
Sweetness intensity	7	25	6	32*	1	31
Consistency	2	3	1	1	3	4
Acceptability	9	7	16	16	7	23
b)	**SK2-SK3**	**SK2-SK15**	**SK2-SK16**	**SK3-SK15**	**SK3-SK16**	**SK15-SK16**
Colour	9	0	7	9	2	7
Colour intensity	21	13	10	34*	31	3
Taste	24	15	11	9	35*	26
Taste intensity	1	24	11	34*	1	35*
Sweetness	40*	21	14	19	26	7
Sweetness intensity	11	35*	25	46*	36*	10
Consistency	16	24	11	8	5	13
Acceptability	10	18	12	8	22	30
c)	**K2-SK2**	**K3-SK3**	**K15-SK15**	**K16-SK16**		
Colour	14	7	18	6		
Colour intensity	16	11	9	4		
Taste	2	33*	4	25		
Taste intensity	10	6	36*	7		
Sweetness	27	31	14	8		
Sweetness intensity	35*	53*	25	16		
Consistency	20	2	7	10		
Acceptability	11	8	0	7		

*significant differences at the 0.05 level according Tukey HSD

Table 6. Correspondence analysis. Contribution of the two dimensions explaining most of the variability of the results of the sensory analysis, to inertia of attributes and ketchup samples.

Attribute/ketchup	D1	D2	Total
Colour	0.591	0.101	0.692
Colour intensity	0.876	0.024	0.900
Taste	0.582	0.266	0.848
Taste intensity	0.504	0.144	0.648
Sweetness	0.187	0.512	0.699
Sweetness intensity	0.712	0.272	0.985
Consistency	0.032	0.857	0.888
Acceptability	0.012	0.748	0.760
K2	0.366	0.256	0.622
K3	0.499	0.184	0.683
K15	0.453	0.208	0.660
K16	0.683	0.134	0.817
SK2	0.059	0.691	0.750
SK3	0.980	0.007	0.987
SK15	0.214	0.589	0.803
SK16	0.134	0.559	0.693

Implications for gastronomy

Following the World Health Organization recommendations about healthy diet where energy intake and sugar content should be reduced controlling dietary intake and therefore, curbing obesity rates, the use of alternative ingredients for producing ancient recipes as ketchup can result in interesting modifications in quality as well as nutritional and sensory characteristics. Sweeteners allow to meet those requirements and provide the mildness expected in some type of foods and algae have been used historically as a foodstuff, even as a delicacy or ancestral food in cultures around the world. Different types of algae have entered the international cuisine due to their nutritional value and have found many applications where the consumption, day by day, has been growing. In Spain recognized chefs as Ángel León were pioneers in the use of microalgae in haute cuisine, using strains locally produced. However, microalgae are still scarcely considered in haute cuisine and it would be interesting to introduced them for dinner guests thought gastronomy, since it offers the possibility to study food and culture to obtain and test new food developments focusing on gourmet cuisine. Microalgae has high protein content and is a rich source of vitamins, fatty acids, minerals and pigments, providing many nutrients to the human being and simultaneously, colour and versatility to mix with other ingredients or side dishes. They have been used for the development of soups, emulsions, beverages and snacks so far, using from convectional techniques (e.g., kneading, baking) to more innovative techniques as extrusion and 3D food printing.

Conflicts of Interest

The authors 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.

Author contributions

P. G. and J. M.: designed the study; Z.N.U-W., R.G., C.S. and M. I., conducted the study; P. G. and M.I. performed statistical analysis; Z.N.U-W., M.I. and J. M. wrote the manuscript and had primary responsibility for the final content. All authors have read and approved the final manuscript.