A chemometric approach to evaluate the impact of pulses, Chlorella and Spirulina on proximate composition, amino acid, and physicochemical properties of turkey burgers

Running title: Addition of pulses, Spirulina or Chlorella alters turkey burgers properties

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Abstract

Background: Changes in physicochemical parameters, proximate composition, amino acid and taste profiles of turkey burgers enriched at 1% with soy (control), pulses, Chlorella and Spirulina proteins were studied.

Results: Color parameters, pH, ash content, total, essential and non-essential amino acids were significantly different among the different type of turkey burgers prepared. In this regard, turkey burgers made with pea protein presented the highest values for pH and lightness, whereas the samples prepared with broad bean showed the highest redness. The inclusion of bean and seaweed produced a marked increase of glutamic acid, lysine and aspartic acid. However, the taste profile was similar in the different six turkey burgers studied (soy, pea, lentil, broad bean, Chlorella and Spirulina protein). Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) allowed to classify turkey burgers according to protein sources, as compared to soy (control). Textural parameters, moisture and color were found to be the most discriminant parameters, able to describe the differences among burgers. Nonetheless, according to the supervised OPLS model, broad beans were found to possess a similar profile to soy (control).

Conclusion: Considering all studied parameters, the enrichment of turkey burgers with bean proteins could be used as a promising alternative to soy proteins from a technological point of view.

Keywords: Textural properties; color parameters; seaweeds; taste profile; turkey burger

1. Introduction

Among foods of animal origin, it is known that meat and its derivatives are a good source of nutrients, among which we can highlight vitamins such as B_{12}, minerals such as iron and zinc, and lipids and proteins[1-3]. This makes meat products an important group of foods which is also consumed globally by a large number of people. But, despite the nutritional value of its components, an excessive consumption of meat, specially processed meat, is related to the risk of cancer development, being colon cancer the most remarkable[4,5]. Several processed meat products incorporate soy proteins, due to their beneficial properties for health, and their good nutritional profile, with a large number of essential amino acids[6,7]. However, soy is a recognized allergen, so it is suitable to look for other vegetable sources of good nutritional quality to replace it.

Legumes, which include lentils, peas and beans, are rich in protein, since they represent 20% of dry weight in the case of beans and peas[8]. Furthermore, products derived from algae are now becoming important[9]. Microalgae are a good source of polyunsaturated fatty acids (PUFA), especially DHA and EPA, antioxidants (phenolic acids and flavonoids), polysaccharides and proteins[10-13]. For all these components of great nutritional relevance and bioactive nature, algae have been used both in the food industry (to make food supplements and nutraceuticals) and in the cosmetic industry[14-16]. The use of these sources has allowed food improving such as nutritional value or oxidation of lipids among others[17-19].

Another factor to take into account is the environmental impact. As it is known, meat production has a high ecological cost, and is contributing to climate change[20]. In contrast, plants with a high protein value such as soybean, pea or lentil, and also algae have a lower land use footprint, lower water use footprint and lower carbon footprint[21].

In addition, legumes can fix atmospheric nitrogen, which would reduce the use of nitrogen fertilizers[21].

Focusing on the food industry, algae have been used for several purposes such as improving the quality of meat and eggs by using them in feeding animals or using them to reduce cholesterol levels[15,22]. In addition, microalgae contain important amounts of essential amino acids and proteins, thus constituting a good alternative source of protein[15, 22-25]. It has been seen that these proteins are of high quality, comparable to others of plant origin such as rice, wheat or beans[10, 26,27].

Many algae have been studied in relation to their protein content. For example, Milovanović et al.[28] noticed that several strains of cyanobacteria have a high protein content (42.8% to 76.5% on a dry weight basis). One of these algae, *Spirulina*, has been found to have protein levels comparable to soy and even meat, so its use in food would be profitable for health, due to its composition of amino acids, polyphenols, essential fatty acids, vitamins and minerals[29].

Incorporating new ingredients into processed foods can alter characteristics such as taste. In this sense, it is known that some amino acids provide the sweet taste (glycine, threonine, alanine, serine and proline), others, a bitter taste (phenylalanine, histidine, leucine, isoleucine, alo-isoleucine, methionine, valine and tryptophan), and finally some of them are responsible for the umami taste (glutamic acid and aspartic acid)[30]. All these considerations have to be taken into account when adding *Chlorella*, *Spirulina* or other vegetable proteins to processed food.

On the other hand, the term "chemometrics" describes the statistical and mathematical approaches used to optimize the design of experiments and extract useful information from large and complex datasets[31]. Chemical data commonly include values and properties of various compounds determined by laboratory experiments and having numerous sources of variance[32]. Usually, chemometrics is applied when there is a large and complex dataset, in terms of sample numbers, types, and responses. The results are used for authentication of geographical origin, farming systems, or even to trace adulteration of high value-added commodities[32].

The aim of this work was to determine the changes in the physicochemical properties and nutritional quality of turkey burgers adding different protein sources of vegetable origin (peas, lentils and beans) and algae (*Chlorella* and *Spirulina*) to turkey meat, and to compare them with soy protein, which was used as a control due to its wide use in the industry.

2. Material and Methods

2.1. Experimental design and manufacture of turkey burger

Novafrigsa S.A. – Grupo Coren (Lugo, Spain) provided the turkey meat. All the additives and spices used were of food grade. Vitessence™ Pulse Proteins supplied soy, pea, lentil and bean proteins. Algaenergy (Madrid, Spain) supplied *Chlorella* and *Spirulina* proteins. All the chemicals used for the analysis were of analytical grade.

A total of 30 turkey hamburgers, in six separated batches, were made in Meat Technology Center (Galicia, Spain), 5 with each protein source. In order to do so, the turkey lean meat was grinded with a refrigerated mincer machine (La Minerva, Bologna, Italy), and it was vacuum-minced with all the additives but the protein extract in a vacuum mincer machine (Fuerpla, Valencia, Spain). Then it was divided in 6 batches, one for each protein source, the designed protein was added at 1% and was cold stored (4 ºC) for 4h. After all that, the burgers were elaborated using a burger-maker (Gaser, A-2000, Girona, Spain).

2.2. Physicochemical parameters

Physicochemical parameters were analyzed according to the method previously reported by Lorenzo et al.[33].The pH of burgers was measured using a digital portable pH-meter (HI 99163, Hanna Instruments, Eibar, Spain) equipped with a penetration probe. Color was measured using a portable colorimeter (CM-600d-Konica Minolta, Japan) with pulsed xenon arc lam, 0^{o} viewing angle geometry and 8 mm aperture size, to estimate burger color in the CIELAB space: lightness, ($L*$); redness, ($a*$); yellowness, ($b*$).The color was measured in three different points of each sample in homogeneous and representative areas, free of fat.Water-holding capacity (WHC) was measured as cooking loos (%), whereas textural profile analysis (TPA) test was conducted using a texture analyser (TA-XT2, Stable Micro Systems, Godalming, UK)according to the method proposed by Lorenzo & Carballo (34) using a load cell of 5 Kg. Hardness (N), adhesiveness (g·s), elasticity (mm), cohesiveness, gumminess (N) and chewiness (N × mm) were obtained using Texture Exponent 32 software (version 1.0.0.68, Stable Micro Systems, Vienna Court, UK).

2.3. Proximate composition

To determinate moisture content, a sample was dried at 105 ºC until achieve constant weight and then weight loss was measured, according to ISO 1442:1997[35]. For protein determination, Kjeldahl total nitrogen method was used with a nitrogen conversion factor of 6.25 for proteins[36]. Lipid extraction was performed submitting samples to a liquid-solid extraction employing petroleum ether in an extractor apparatus (AnkomHCI Hydrolysis System, USA) at 90 ºC during 60 min, following the AOCS official method Am 5-04. Last, ash content was calculated by maintaining the sample at 600 ºC in a muffle furnace (Carbolite RWF 1200, Hope Valley, England) until constant weight, according to ISO 936:1998[37].

2.4. Amino acid content

Hydrolyzed amino acid composition (g/100 g of meat) of manufactured turkey burgers was estimated using the procedure previously described by Lorenzo et al.[38]. 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQ-Fluor reagent kit) was used for amino acids derivatization, which were determined by RP-HPLC (Waters 2695 Separations Module+Waters 2475 Multi Fluorescence Detector+WatersAccQ-Tag amino acids analysis column).

2.5. Statistical analysis

One-way ANOVA was used to examine the results obtained. Furthermore, Duncan's test was made to compare the means. To determine the coefficients which maximize the differences among samples, a linear discriminate function containing an optimal subset of variables was used. Data were presented as mean ± standard deviation (SD) of five replicates, and was considered significant when $p<0.05$. All statistical analyses were performed using the software Statgraphics Centurion XVI.I® (Statgraphics Technologies, Virginia, USA).

Afterwards, the whole dataset was imported into SIMCA 13 (Umetrics, Malmö, Sweden), UV scaled, and elaborated by means of orthogonal projections to latent structures discriminant analysis (OPLS-DA) supervised modelling. In this regard, the variation between the groups was separated into predictive and orthogonal components (i.e., ascribable to technical and biological variation). The OPLS-DA score plot allowed describing similarity/un-relatedness between treatments and to identify the parameters better depicting differences. To support and validate the plot observed, the presence of outliers was inspected by means of Hotelling's T2, using 95% and 99% confidence limits for suspect and strong outliers, respectively. Furthermore, the goodness-of-fit (R^2Y) and the goodness-of-prediction (Q^2Y) were also considered, using a threshold value of > 0.5for the latter. Cross-validation (CV-ANOVA; $P<0.01$) and permutation testing (N = 100) were then carried out to validate and exclude overfitting. Finally, the VIP (i.e., variable importance in projection) selection method was used to select those parameters possessing the highest discrimination potential, setting a VIP score > 1.

3. Results and Discussion

3.1 Physicochemical parameters

The changes in pH (Fig. 1A) and color parameters (Fig. 1B) when adding proteins from different sources are shown in **Figure 1**. Slight variations in pH were found, however the statistical analysis revealed that there were significant differences ($P<0.05$) among samples, with the highest pH value corresponding to the sample made with soy protein (6.38). These results contrast to the results of Parniakov et al.[39], since in their study, the highest pH values found in chicken rotti prepared with *Chlorella* and *Spirullina* proteins. However, Cofrades et al.[40] also obtained lower pH values when *Himanthalia elongata* was incorporated to meat products.

Besides, in the color parameters, it can be seen that the highest L* value belongs to the legume proteins, while the algae (*Chlorella* and *Spirulina*) presented the lowest ones. This is also the case with a*, where the negative value of the algae may be due to their green and blue pigments. Finally, b* values ranged between 8.11 and 17.72, in turkey burgers prepared with *Spirulina* protein, which showed the lowest value, while the samples elaborated with pea protein presented the highest one. This matches completely with the results obtained by Parniakov et al.[39], who obtained similar results in chicken rotti manufactured with different protein source. On the other hand, water holding capacity (WHC) was not altered by the incorporation of different vegetable proteins to turkey burgers.

3.2 Proximate composition

Since the formulation for the elaboration of each one of the lots is the same, with exception of the protein used, one would not expect large changes in its chemical composition. The same is observed in **Figure 2**, where neither the moisture nor the lipid content showed significant differences among batches. However, the protein content is different, with the maximum value corresponding to the samples prepared with soy protein (15.44%) and the minimum value for turkey burgers elaborated with *Chlorella* protein (14.81%). The percentage of ashes also changed among the different batches. However, the interval of the values is much narrower, since the minimum value was obtained in the batch elaborated with lentil protein (2.00%) and the maximum one corresponds to the batch prepared with the *Spirulina* protein (2.05%). As already mentioned above, these ranges of such small values are due to the fact that the formulation is exactly the same in all batches, with the exception of the protein added, which only represents 1% of the final composition.

Neither did Parniakov et al.[39] obtained significant changes in the percentage of moisture. However, they found changes in the lipid content, obtaining a maximum value of 5.82% in the chicken rotti batch made with *Chlorella* protein. In contrast to our study, both protein and ash contents were higher in the batch of lentil protein: 22.06% and 3.22% respectively.

On the other hand, López-López et al.[41] obtained a reduction in moisture and lipid content by adding *Himanthalia elongata* to frankfurters, and comparing it with the control sample, containing soy. They also observed an increase in the amount of protein and ash in the products enriched with algae compared to the control .Finally, Cofrades et al.[40] noticed a decrease in moisture and ash content by adding *Porphyraum bilicalis* or *Himanthali aelongata* to meat food.

3.3 Textural parameters

Regarding textural properties, only elasticity and adhesiveness showed significant differences among groups (**Figure 3**). Elasticity was higher in samples prepared with soy protein (0.61 mm) than in the other samples. Furthermore, adhesiveness showed its highest value in burgers made with lentil protein (-17.04 g·s), whereas the lowest results were obtained in burgers made with *Chlorella* and *Spirulina* proteins, very close to 0 (-0.66 and -1.08 g·s, respectively). Other studies such as Choi et al.[42] presented a reduction in parameters such as hardness, chewiness or gumminess when adding *Laminaria japonica* to pork patties. Moreover, Parniakov et al.[39] observed a decrease in textural parameters with the exception of adhesiveness in chicken rotti containing *Chlorella* or *Spirulina* proteins. However, authors such as Cofrades et al.[40] or López-López et al.[41] noticed an increase in the parameters of hardness and chewiness by adding *Himanthalia elongata* to meat gel/emulsion or Frankfurt sausages.

3.4 Amino acid content

Table 1 shows the influence of addition of different proteins of vegetable and microalga origin in turkey burgers. The hydrolyzed amino acid profile of turkey burgers included 17 out of 20 amino acids constituting food proteins. Arginine was included in the essential amino acid group[43]. Cysteine and methionine were not detected. It can be seen that amino acids such as serine, glycine, arginine, and tyrosine did not show significant changes. In all these cases, the minimum value corresponded to samples prepared with pea protein, while the maximum values correspond to turkey burgers elaborated with *Spirulina* and broad bean proteins. As for the rest of amino acids, glutamic acid was the predominant, obtaining a maximum value of 2.21 g/100 g in the case of broad bean protein. This is interesting from the sensory point of view, since it is known that glutamic acid provides umami flavor[44]. It is also observed that histidine was the minority amino acid, with a minimum value of 0.25 g/100 g for the pea protein. The amino acids with a greater difference among the different batches were glutamic acid, valine, lysine, isoleucine, leucine and phenylalanine. These results agree with the data obtained by Cofrades et al.[45] who observed an increase in these amino acids when adding *Porphyraum bilicalis* to meat emulsions.

On the other hand, the batch of turkey burgers with a higher total amino acid content was the one with *Spirulina* added, as well as a ratio between essential and non-essential amino acids close to 1. However, the total amount of lower amino acids belongs to burgers prepared with pea protein, while the worst ratio of essential/non-essential amino acids was found in samples elaborated with soy protein. In this sense, Parniakov et al.[39] also found the highest total amino acid content in chicken rotti made with *Chlorella* and *Spirulina* proteins and the lowest in samples prepared with pea proteins. In addition, these authors obtained a ratio of more than 1 essential/non-essential amino acids for *Chlorella* and *Spirulina* proteins, which showed that both algae are a good source of essential amino acids. López-López et al.[41] and Dawczynski et al.[46] also found an increase in the total amount of amino acids by incorporating algal proteins into meat products. Therefore, this study suggests that soy protein in meat products can be replaced by broad bean protein or algae such as *Chlorella* or *Spirulina*, thus improving the protein profile of the food.

3.5. Chemometric evaluation

The orthogonal projection to latent structure discriminant analysis (OPLS-DA) was used as supervised statistical tool to plot similarities/differences among turkey burgers on the basis of all the parameters studied. This multivariate data analysis allows extracting information from complex dataset characterized by multiple variables and using all the variables simultaneously. In particular, OPLS models are able to rotate the projection so that the model focuses on the effect of interest, thus separating data into predictive and uncorrelated information (i.e., orthogonal signal correction). The dataset based on physicochemical parameters (pH, color and texture), proximate composition (moisture, protein, lipid and ash content), and amino acids profile was used for this statistical analysis.

According to our experimental conditions, two OPLS model were considered. In the first model, the protein source (soy, broad beans, lentils, beans, *Chlorella* and *Spirulina*) was used as class membership criteria, in order to identify those turkey burgers more similar according to the parameters aforementioned. The OPLS score plot obtained by means of the latter considerations is reported in **Figure 4**.

Interestingly, the separation between groups was very good with beans and lentils proteins possessing a very similar profile and clustering together. A similar information was obtained looking at soy and broad beans proteins, that were included in the same region of the OPLS score plot. Notably, *Chlorella* and *Spirulina* proteins i.e., those burgers enriched with microalgae, were completely different in terms of the parameters studied, when compared to the other turkey burger samples. The OPLS model obtained suggested that, among pulses, broad bean was the protein source that allow to obtain a burger similar to the control (soy). Afterwards, the variable selection method VIP (variable importance in projection) was used to identify those parameters allowing the score plot hyperspace distribution previously described. In this regard, the most discriminating parameters possessing a VIP score > 1 are reported in **Table 2**.

As a general consideration, 14 discriminating parameters were identified, with textural parameters such as cohesiveness, elasticity and adhesiveness possessing a very high discrimination potential, followed by those related to color (L^*, a^*, and b^* values).

Interestingly, other parameters such as chewiness and hardness were found to be very important, thus confirming that the final texture was very affected by the alternative protein replacement. The OPLS model was cross-validated by means of permutation testing (N = 100) and inspected for outliers by means of Hotelling's T2 range (supplementary material), then checking model parameters that were found to be more than acceptable, being $R^2Y = 0.85$ and $Q^2Y = 0.63$.

A second OPLS model was carried out to discriminate the six turkey burgers prepared according to the 'type of protein' (i.e., control *vs.* pulses and microalgae). The OPLS score plot obtained with this second interpretation is reported in **Figure 5**.

This analysis allowed confirming that pulse proteins were the protein source most similar to the control (soy) in comparison to microalgae. Indeed, the second latent vector of the OPLS space clearly discriminated *Chlorella* and *Spirulina* burgers from the other ones. This second OPLS model was cross-validated and checked again for outliers (supplementary material), and provided very robust fitting parameters ($R^2Y = 0.85$ and $Q^2Y = 0.70$). Afterwards, the VIP selection was used again to identity those parameters differentiating the classes of protein sources (**Table 3**).

Interestingly, a lower number of variables possessing a VIP score > 1was obtained (i.e., 7), with color (L^*, a^*, and b^* values) and pH being the most discriminant contributors. This indicates that the enrichment of turkey burgers with alternative proteins could affect the visual acceptance, in turn driving the choices by the final consumer. In this regard, as stated by Shan et al.[47], health-oriented reformulations of processed meat are very promising in terms of addressing increasing health concerns regarding this food category; however, consumer acceptance cannot be taken for granted[47].

4. Conclusions

The use of different vegetable and microalgae proteins as soy protein replacers in the preparation of turkey burgers with improved nutritional profile, produces changes, both in the physicochemical properties and protein profile. Burgers prepared with *Spirulina* and broad bean protein presented the highest amount of total amino acids. In addition, the ratio between essential and non-essential amino acids increased, indicating that these proteins are a good source of essential amino acids. The color parameters were also greatly altered by *Spirulina* and *Chlorella* proteins, since they were significantly decreased, that shows it has acquired a green-blue color, probably due to the pigments they contain. Finally, there was also observed a reduction in the elasticity and adhesiveness, as well as the pH of the samples with these proteins. The OPLS-DA multivariate modelling carried out from physicochemical, textural and composition parameters suggested that addition of bean and lentil proteins provided a very similar profile, whereas addition of broad bean proteins resulted in a profile very close to soy (control). However, microalgae as proteins source showed the most distinctive and characteristic profiles. Among others, textural parameters (cohesiveness, elasticity and adhesiveness) followed by color (L^*, a^*, and b^* values) possessed the highest discrimination potential. Overall, the choice of a protein source rather than another distinctively affected both textural properties and color.

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Table 1. Amino acid content (expressed as g/100 g) of turkey burgers with different protein replacements by pulses and algae proteins (n=5).

Amino acid	***Protein source***						**Sig.**
	Soy	**Pea**	**Lentils**	**Broad beans**	***Chlorella***	***Spirulina***	
Asp	0.99±0.09^{ab}	0.79±0.14^{a}	0.85±0.14^{a}	1.20±0.23^{b}	1.00±0.08^{ab}	1.14±0.15^{b}	**
Ser	0.43±0.26	0.41±0.05	0.45±0.09	0.58±0.09	0.48±0.04	0.57±0.05	n.s.
Glu	1.77±0.12^{a}	1.46±0.27^{a}	1.56±0.24^{a}	2.21±0.40^{b}	1.85±0.14^{ab}	2.13±0.25^{b}	***
Gly	0.63±0.17	0.48±0.05	0.45±0.08	0.65±0.10	0.51±0.07	0.60±0.06	n.s.
His	0.30±0.03^{a}	0.25±0.04^{a}	0.28±0.06^{a}	0.37±0.05^{b}	0.31±0.03^{ab}	0.35±0.03^{b}	**
Arg	0.86±0.09	0.74±0.11	0.80±0.16	0.88±0.45	0.95±0.05	1.17±0.11	n.s.
Thr	0.51±0.06^{ab}	0.42±0.07^{a}	0.47±0.10^{a}	0.60±0.09^{b}	0.51±0.03^{ab}	0.60±0.05^{b}	*
Ala	0.72±0.12^{bc}	0.56±0.06^{ab}	0.57±0.10^{a}	0.80±0.14^{c}	0.69±0.04^{abc}	0.80±0.10^{c}	*
Pro	0.59±0.11^{bc}	0.47±0.06^{ab}	0.45±0.10^{a}	0.57±0.08^{abc}	0.51±0.02^{abc}	0.61±0.04^{c}	*
Cys	-	-	-	-	-	-	-
Tyr	0.47±0.08	0.36±0.09	0.42±0.15	0.52±0.10	0.39±0.01	0.49±0.06	n.s.
Val	0.52±0.06^{ab}	0.42±0.07^{a}	0.44±0.08^{a}	0.61±0.10^{bc}	0.54±0.02^{abc}	0.62±0.06^{c}	***
Met	-	-	-	--	-	-	-
Lys	0.99±0.06^{a}	0.83±0.14^{a}	0.90±0.14^{a}	1.30±0.28^{b}	1.07±0.07^{ab}	1.22±0.16^{b}	***
Ile	0.53±0.03^{a}	0.43±0.08^{a}	0.45±0.08^{a}	0.64±0.10^{b}	0.55±0.02^{ab}	0.65±0.07^{b}	***
Leu	0.89±0.05^{a}	0.73±0.13^{a}	0.76±0.13^{a}	1.08±0.17^{b}	0.94±0.05^{ab}	1.09±0.11^{b}	***
Phe	0.45±0.04	0.38±0.06	0.41±0.09	0.55±0.07	0.48±0.02	0.55±0.05	***
Total	10.66±0.81^{a}	8.74±1.37^{a}	9.26±1.66^{a}	12.53±1.56^{b}	10.73±0.69^{ab}	12.60±1.25^{b}	***
E	5.07±0.38^{a}	4.21±0.67^{a}	4.51±0.82^{a}	6.02±0.46^{b}	5.31±0.30^{ab}	6.26±0.61^{b}	***
NE	5.59±0.48^{ab}	4.53±0.69^{a}	4.76±0.85^{a}	6.51±1.13^{b}	5.42±0.39^{ab}	6.34±0.64^{b}	**
E/NE	0.91±0.05	0.93±0.01	0.95±0.02	0.94±0.09	0.98±0.01	0.99±0.01	n.s.

E: essentials amino acids; NE: non essential amino acids.All the data are expressed as mean ± standard deviations. Means with the different superscript letters in a row are differ significantly. Sig: Significance; *** (P<0.005). ** (P < 0.01). * (P < 0.05). n.s.: not significant.

Table 2. VIP (variable importance in projection) selection method to identify those parameters discriminating burgers according to the protein source (i.e., soy, broad beans, lentils, beans, *Chlorella* and *Spirulina*).

Variable	**VIP score**	**Standard Error**
Cohesiveness	1.25667	0.677421
Elasticity (mm)	1.20848	0.223379
% Moisture	1.20547	0.407983
Adhesiveness (g.s)	1.16958	0.139647
pH	1.16599	0.351083
b*	1.12919	0.397432
L*	1.11402	0.179352
a*	1.11217	0.168263
% Fat	1.09554	0.556257
Chewiness (N.mm)	1.08377	0.251971
Hardness (N)	1.04924	0.560976
% Protein	1.03569	0.299671
% WHC	1.01428	1.19757
Gumminess (N)	0.964638	0.467577
% Ash	0.951902	0.440015
Gly	0.910074	0.275342
Pro	0.906318	0.265215
Lys	0.881883	0.330559
Ser	0.881457	0.265732
Thr	0.880821	0.268914
Glu	0.878199	0.292486
Ala	0.877588	0.294838
Asp	0.877331	0.307914
Arg	0.875491	0.35891
Val	0.874921	0.264664
His	0.87435	0.279875
Ile	0.874288	0.251043
Leu	0.873122	0.257744
Phe	0.872906	0.249869
Tyr	0.858216	0.245838

Table 3. VIP (variable importance in projection) selection method to identify those parameters discriminating burgers according to the ‘type of protein’ (i.e., control *vs.* pulses and microalgae).

Variable	VIP score	Standard Error
L*	1.74725	0.507909
a*	1.72648	0.589273
pH	1.57491	1.04779
b*	1.39601	0.642895
Elasticity (mm)	1.26783	0.492545
Adhesiveness (g.s)	1.2546	0.550322
% Protein	1.13053	0.474078
Gly	0.956316	0.423798
Pro	0.950281	0.353726
Ala	0.891026	0.284986
Val	0.866575	0.225304
Tyr	0.866432	0.299584
Ile	0.856226	0.19837
Leu	0.853151	0.192762
Asp	0.852202	0.200481
Thr	0.849875	0.223605
Phe	0.84676	0.200456
His	0.843147	0.210289
Glu	0.841142	0.18419
Cohesiveness	0.83058	0.760239
Lys	0.825743	0.181145
Arg	0.815714	0.220525
% Ash	0.777262	0.486084
Gumminess (N)	0.772585	0.749919
Chewiness (N.mm)	0.761671	1.39904
Ser	0.732158	0.129513
% Moisture	0.651411	0.668345
Hardness (N)	0.649502	0.543268
% WHC	0.625979	0.572184
% Fat	0.572034	0.797117

Figure 1. pH (A) and colour parameters (B) of turkey burgers prepared with different proteins (n=5). Data were presented as mean ± standard deviation (SD). Bars with the different letters differ significantly (*P*<0.005)

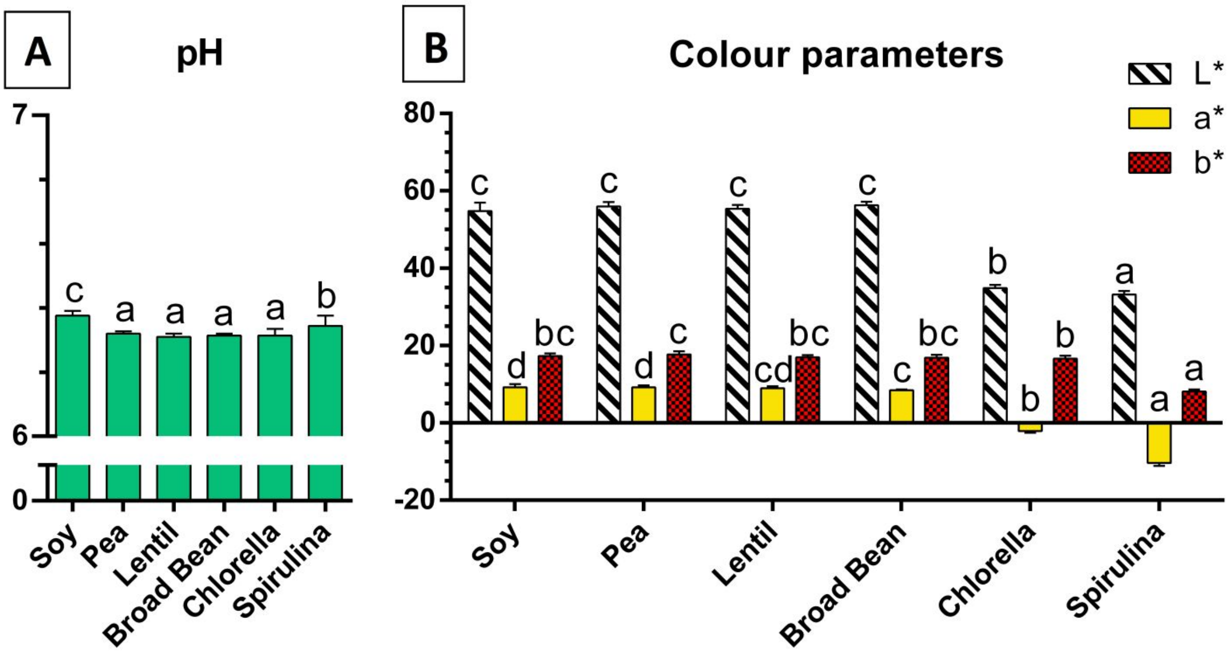

Figure 2. Chemical composition of turkey burgers prepared with different proteins (n=5). Data were presented as mean ± standard deviation (SD). Bars with the different letters differ significantly (*P*<0.05)

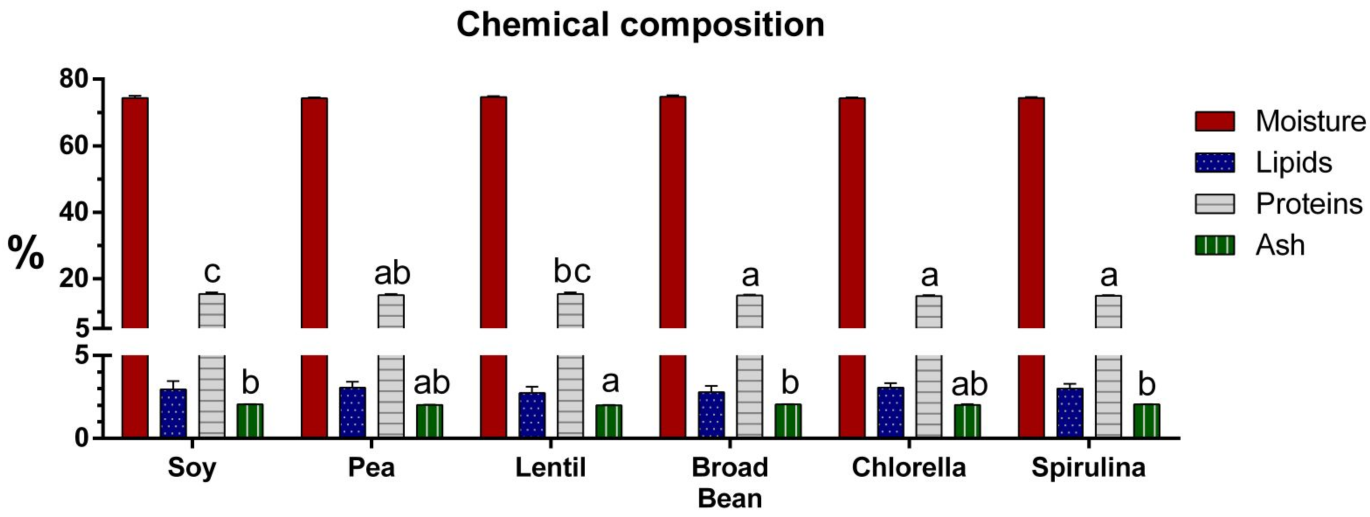

Figure 3. Textural parameters of turkey burgers prepared with different proteins (n=5). Data were presented as mean ± standard deviation (SD). Bars with the different letters differ significantly (*P*<0.05 for Elasticity; *P*<0.005 for Adhesiveness).

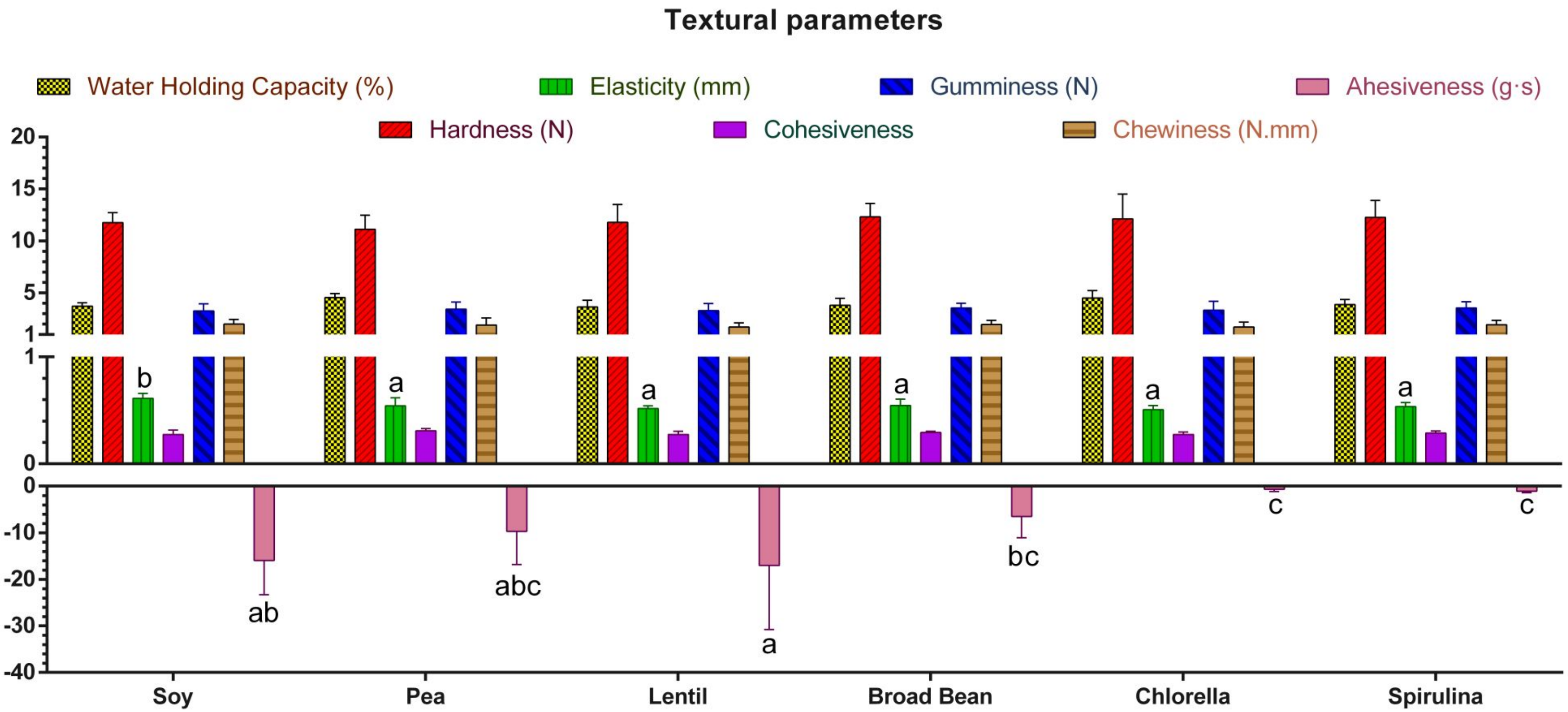

Figure 4. OPLS score plot obtained using the protein source (i.e., soy, broad beans, lentils, beans, *Chlorella* and *Spirulina*) as class membership criteria, in order to identify those turkey burgers more similar

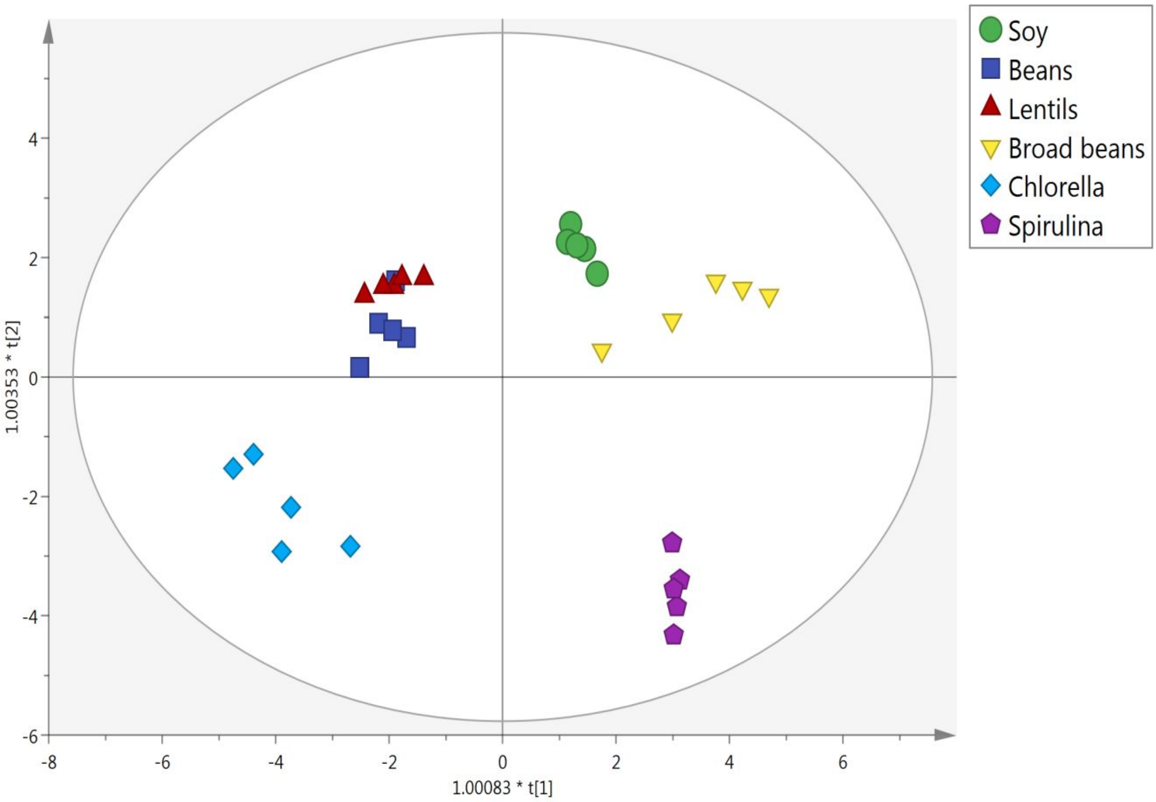

Figure 5. OPLS model to discriminate the six turkey burgers prepared according to the 'type of protein' (i.e., control *vs.* pulses and microalgae)