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High-pressure processing and sous-vide cooking effects on physicochemical properties of meat-based, plant-based and hybrid patties



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ARTICLE INFO

Keywords: High-pressure processing Vacuum-cooking Veal Legume Meat analogue

ABSTRACT

Modern technologies such as high-hydrostatic pressure processing (HPP) and sous-vide cooking (SVCOOK) have not been fully assessed for improving the quality of veal patties. The effects of HPP alone or combined with SVCOOK technique on physicochemical characteristics of veal, plant-based, and hybrid patties were investigated. Samples of the different formulations were subjected to three pressures (350–600 MPa) for 5–15 min, followed by SVCOOK (55–65 °C for 15 min). The color of the HPP treated plant-based and hybrid patties tended to be of less reddish color tone and conformed more towards a yellowish shade. The dual technology treated hybrid patties were like veal patties in color and texture parameters, whereas the physicochemical parameters of plantbased patties were different from veal and hybrid patties. Conversely, the effect of HPP on hybrid patties was not comparable to veal patties. The dual (HPP–SVCOOK) technology has the potential to develop novel hybrid products with physicochemical characteristics comparable to those of veal-based patties.

1. Introduction

The current food industry requires novel technologies to obtain nutritious and environmentally friendly foodstuffs (Curtain & Grafenauer, 2019). The dietary behavior of consumers is constantly evolving. Replacing animal protein with alternative sources has been proposed as a strategy to reach global sustainability (de Boer & Aiking, 2021; Tilman & Clark, 2014). There is an increasing acceptance of flexitarian diets in developed countries (Grasso & Jaworska, 2020; Parlasca & Oaim, 2022). Legumes, algae, and insect proteins have been proposed as meat and fish replacers (Asgar, Fazilah, Huda, Bhat, & Karim, 2010; Verbeke, 2015). A plethora of formulations with wheat gluten, soy protein, rice and lentil has been reported for developing plant-based, protein-rich products (Ahmad et al., 2022). Among them, soy-based tempeh and tofu have gained the greatest acceptance worldwide (Ahmad et al., 2022). The migration of meat-based diets to plant-based diets and the increasing acceptability of hybrid products have been recently acknowledged (European Commission, 2020; Grasso, Asioli, & Smith, 2022). Consumer studies have suggested that rather than eliminating meat, a diet with a moderate amount of meat should be encouraged (Corrin & Papadopoulos, 2017; Grasso & Jaworska, 2020). A hybrid product with half meat protein and half plant-based protein can be more appealing to the flexitarian community and, at the same time, combines the potential nutritional benefits of both ingredients. Since most of the plant proteins are known to be low in lysine, cysteine, and methionine (Hertzler, Lieblein-Boff, Weiler, & Allgeier, 2020), blending them with meat improves their nutritional value. The complete shift to plant-based diets has always been challenging, as they are perceived as unfamiliar and unappealing. Hybrid products could contribute to this shift as they still adhere to the current meat hierarchy occupying a central role (Lang, 2020). An example of new hybrid product is the burger created by Chandler and McSweeney (2022), which combine ground chicken with chickpea, lentil, and pea flours.

Keeping up with the increased demand and the changing mindset of consumers, the development of new sustainable products with enhanced shelf life and an acceptable quality level is necessary. Emerging technologies such as high-hydrostatic pressure processing (HPP) and sousvide cooking (SVCOOK) have not been fully assessed for improving the quality of veal patties.

HPP has improved food safety, textural quality, and technological properties of various meat and vegetable products (Das, Sharma, & Sarkar, 2022; Ding et al., 2021; Gómez, Janardhanan, Ibañez, & Beriain, 2020; Mulla, Subramanian, & Dar, 2022). Changes in textural and technological properties of meat and pulses are attributable to structural

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https://doi.org/10.1016/j.lwt.2022.114273

Received 24 June 2022; Received in revised form 27 November 2022; Accepted 4 December 2022 Available online 9 December 2022

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modifications of their proteins (Bolumar et al., 2021; Ding et al., 2021).). SVCOOK is a low-temperature, water bath cooking technique where the product is previously vacuum packaged. SVCOOK preserves the flavor and provides a pleasant, unique texture to the meat product (Gómez et al., 2020).

Even though there is an increasing demand for products formulated with alternative sources of protein, there is not yet enough information about their compositional (proximate), textural, color, and other physicochemical traits, nor when patties are made with veal or when veal is combined with plant ingredients to produce a hybrid product. Also, the effects of existing technologies such as HPP and (or) SVCOOK on conventional meat patties and their analogs must be examined to determine if they elicit favorable physicochemical changes. A previous study on HPP and SVCOOK processed veal patties (Janardhanan, Virseda, Huerta-Leidenz, & Beriain, 2022) suggested a beneficial effect on the texture and color. It was hypothesized that a combination of HPP and SVCOOK would elicit a beneficial, synergistic effect on the physicochemical properties of veal-based, plant-based and hybrid products. The main objective of this study is to investigate the differential response of veal-based, plant based and hybrid patties to the single or combined effects of HPP and SVCOOK in terms of their proximate, textural color, and other physicochemical characteristics.

2. Materials and methods

2.1. Sample preparation

All the raw materials were locally procured. The study was conducted on patties of three different formulations (i.e., a meat-based, plant-based product, and a hybrid product). The plant-based ingredient was Legumbreta Fina, a commercially available extruded product made from mixed flours (soy, rice and bean), and was obtained from a local manufacturer (Sanygran SL, Tudela, Spain). The hybrid product was prepared with 50-50 protein mix of meat and plant-based products. The meat (*Biceps femoris*) used for preparing the veal-based and hybrid patties in the study was derived from three bullocks produced under the conditions required by the protected geographical indication Ternera de Navarra (NAVEAL). The experiment complied with the official guidelines for humane treatment, care, and handling of animals (The Council of the European Union, 2009).

The 69 g of the plant-based ingredient were soaked in 81 mL of water to prepare the plant-based patty (150 g), such that the product has similar protein content as NAVEAL. It was consecutively pressed and formed into plant-based patties and packaged in polyamide-polypropylene bags by a chamber vacuum machine (C412 Lerica, Venice, Italy) for further analysis. The external connective tissue and visible fat of the NAVEAL used for the meat-based and hybrid patties were trimmed off; the meat was reduced to cubes and minced at low speed for 20-30 s at 20 °C using a meat grinder machine (CT20 electric meat grinder, Urbiola SL, Noain, Spain). The ground meat (150 g) was pressed into NAVEAL patties between two grease-proof paper sheets. The hybrid patty (150 g) was prepared by mixing both NAVEAL (75 g) and plant-based product (75 g) in such manner that the total protein composition of the product was maintained at 50% from both the animal and plant origin and formed into the patties' shape between two greaseproof paper sheets using a patty press. The samples were then vacuum packaged (98% vacuum) in bags using the chamber vacuum machine (C412 Lerica, Venice, Italy). The samples were then stored at 4 °C for 24 h until they were subjected to HPP.

2.2. Experimental design

The experimental design for Response Surface Methodology (RSM) was performed using a statistical software package (Minitab® vers 19.2020.1, Minitab LLC., State College, PA, USA). A phase-centered central composite design (CCD) was used to determine the design

area. The design area was defined as the corner points and the center points of the set conditions, to reduce the treatment number (Montgomery & Myers, 1995). According to the CCD, the experimental design for HPP was divided into 13 runs, the center point was replicated five times (Appendix Table A1), whereas the design for HPP plus SVCOOK was split into 20 runs with three blocks, and the center point was replicated six times (Appendix Table A2). The NAVEAL and hybrid patties were distinctly divided into three blocks for the HPP plus SVCOOK experiment (Appendix Table A3). One hundred and fifty-nine burger patties were prepared, and thirteen patties from each of NAVEAL, plant-based and hybrid formulations were only subjected to HPP (HPP samples). Forty patties of each kind (NAVEAL, plant-based and hybrid) were exposed to HPP and subsequent SVCOOK (HPP +SVCOOK samples), where one patty in each treatment was used for temperature monitoring. Fig. 1 shows the flowchart for the experimental procedures.

2.3. Treatments

The samples were subjected to various ranges of pressure from 350 to 600 MPa for 5–15 min, according to the RSM-CCD. The samples were exposed to HPP using an Idus 10 L HPP system (Metronics Technologies S.L., Noain, Spain). The device was a 600 MPa limit lab scale model, which works as a real-world industrial machine. The pressurized samples were stored at -18 °C until further analysis or SVCOOK. HPP samples were cooked at a temperature range of 55–65 °C for 15 min, according to the RSM-CCD. A cooking bath (Orved SV Thermo-Top, Orved S.P.A, Venice, Italy) was used for the low-temperature SVCOOK. The resistance temperature detector probes were used to monitor the product's core temperature. Once the core reached the set temperature, it was maintained for 15 min. The cooked samples were stored at 4 °C for 24 h until further analyses.

2.4. Proximate analysis

Moisture (International Organization for Standardization, 1997), protein (International Organization for Standardization, 1978), fat (International Organization for Standardization, 1973), and total ash contents (International Organization for Standardization, 1998) of the hybrid and the plant-based samples along with the flour, as raw material for the plant-based patty, were performed in duplicate.

2.5. Cooking loss

The weights of the individual raw samples before and after SVCOOK were taken, and the cooking loss was calculated using the formula reported by Murphy, Criner, and Gray (1975).

Cooking loss (%) =
$$[(m_b - m_a) \ge 100] / m_b$$
 (1)

where m_b and m_a represent the sample weights before and after cooking, respectively.

2.6. pH

The pH of the HPP samples and the combined HPP + SVCOOK samples were measured in triplicate at 25 °C (International Organization for Standardization, 1999) by means of a pH-meter (Crison Instruments S.A., Barcelona, Spain) with a combined probe electrode. The device was calibrated using pH buffer solutions of pH 4.01 and 7.00 at 25 °C.

2.7. Instrumental color

Color parameter (L^* , a^* , b^*) values of processed samples after the HPP and HPP + SVCOOK respective treatments were collected. The *Chroma* (C^*) and hue angle (h^*) were calculated as follows:



Fig. 1. Experimental design for preparing patties and treatments by high-hydrostatic pressure processing (HPP) and sous-vide cooking (SVCOOK).

$$C * = (a*^2 + b*^2)^{1/2}$$

 $h * = \tan^{-1}(b * / a *)$

A handheld spectrophotometer (Minolta CM-2300d, Konica Minolta Business Technologies Inc., Tokyo, Japan) was used for measuring the color parameters, setting D65 illuminant with a \emptyset 52 mm sphere size, \emptyset 8 mm measurement area, \emptyset 11 mm illumination area, and 10° observer angle. The instrument was zero and white calibrated before use. Six consecutive readings were recorded.

2.8. Instrumental texture

Texture parameters were determined according to the method described by Mittal, Nadulski, Barbut, and Negi (1992). A Texture Profile Analysis (TPA) of HPP and HPP + SVCOOK samples was conducted using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Surrey, UK) fitted with a loadcell of 30 kg. Previously, the apparatus was calibrated with a 2 kg weight. An aluminum cylindrical probe (\emptyset 25 mm, h 35 mm) with a pre-test, test, and post-test speed fixed as 2 mm/s was used. Samples (1.5×1.5 cm) were subjected to a two-cycle 50% compression at room temperature. The compression time was set as 3 s. Data from six consecutive measures were collected with Exponent Lite version 6.1. software (Stable Micro Systems Ltd., Surrey, UK).

2.9. Data analysis and modeling

Descriptive statistics for the physicochemical parameters of the HPP treated and HPP + SVCOOK samples were calculated. ANOVA and *post hoc* Tukey test (5% level of significance) were conducted to compare the mean values of physical parameters according to the formulation.

The effect of the independent factors and their interactions, such as pressure (350–600 MPa), pressurization time (5–15 min), SVCOOK temperature (55–65 °C) on the response variables, were assessed according to the experimental design. A polynomial model using the statistical software (Minitab® version 19.2020.1, Minitab LLC., State College, PA, USA) evaluated the multiple regression of the experimental data corresponding to the responses to the independent variables. The goodness of fit of the models was evaluated by the determination

coefficient (R^2), and the lack of fit was calculated by default. A two-way ANOVA was performed to find out whether the terms and the models were statistically significant (P < 0.05). Stepwise backward elimination with α greater than 0.051 was set for selecting the regression models. The statistically significant model with the highest R^2 was selected as the regression model of the response. The two-sided confidence level for all intervals was set at 95% for all the parameters of the models. The regression models were quadratic, 2-way interaction models and linear models.

A Principal Component Analysis (PCA) (Jolliffe, 2002) was carried out on physicochemical parameters of the samples (plant-based, hybrid, and NAVEAL patties). The rotation of the principal components was accomplished with varimax method to preserve the largest variance explained among the data. Two principal components were retained to reduce the dimensionality of original parameters, and the factor scores for samples were obtained by regression method. The sample scores were plotted together to explore relationships. The PCA was executed using the SPSS Statistics software version 27.0 (IBM Corp., Armonk, NY, USA).

3. Results and discussions

The descriptive statistics and the *post hoc* analysis for parameters of samples are reported in Appendix Tables A4-A9.

3.1. Proximate analysis

The proximate analysis of the hybrid and plant-based samples is summarized in Table 1. Protein and fat contents reached values of around 22% and 1.4%, respectively. Plant-based products manufactured from legume and cereal sources contained higher mean percentages of protein and moderate in fat as compared to meat products (Kaleda et al., 2021). In general, marketed meat analogs contain over 20% protein and under 11% fat. However, these foodstuffs cannot be considered a "short-term" alternative to meat products from a nutritional perspective (Cutroneo et al., 2022).

Table 1

Proximate composition of NAVEAL (N), hybrid (H) and plant-based (P) patties, and ground flour of the raw plant-based ingredient (P flour).

Sample	Moistur	e (%)	Protein	(%)	Fat (%)		Ash (%))
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
N	75.30	0.17	22.47	0.23	1.50	0.15	1.20	0.04
H1	65.22	0.20	22.07	0.05	1.48	0.16	2.23	0.06
H2	65.01	0.09	22.56	0.29	1.31	0.09	2.06	0.03
H3	65.95	0.03	22.02	0.21	1.37	0.17	2.02	0.01
Р	56.42	0.00	22.80	0.15	1.07	0.01	2.93	0.04
P flour	6.15	0.03	47.97	2.23	2.26	0.02	6.46	0.15

H1, H2, H3 = hybrid samples from each block of the experimental design.

3.2. Effect of HPP on physical parameters

3.2.1. Plant-based patties

The results obtained by applying RSM are presented in Table 2. A significant effect of pressure and pressurization time was observed in a^* value for the plant-based patties (P < 0.05). The a^* values were found to increase linearly with the rise in the HPP pressure. Conversely, the a^* value fitted a curvilinear relationship with pressurization time (Fig. 2a). The h^* values (Fig. 2b) were found to follow a similar relationship as demonstrated by the a^* values. The changes in the a^* value reflect the incline of the color towards redness at higher pressures. Similarly, high pressure treatment enhanced L^* but reduced a^* values in chickpeas (Alsalman & Ramaswamy, 2020) which was inferred as a result of the enzymatic reactions. However, in NAVEAL patties, it had been reported that a significant effect of HPP was observed in the b^* value alone (Janardhanan et al., 2022). The increase of yellowness in NAVEAL with the rise in pressure was inferred to be due to the oxidation of ferrous myoglobin.

The a^* and h^* values of the plant-based patties were found to be significantly different (P < 0.05) from the NAVEAL patties. By comparing the color parameters among the three formulations (plant-based, NAVEAL and hybrid), it could be noted that the plant-based samples were similar to hybrid samples. Color components like lycopene, carrot juice and leghemoglobin are added to commercial plant-based patties to mimic the redness of meat patties (Bohrer, 2019).

The pH of plant-based patties exhibited a linear relationship with HPP pressure (P < 0.05) (Table 2). Similar effects of HPP pressure on NAVEAL were previously cited (Janardhanan et al., 2022). The pH mean values of plant-based samples were less acidic and differed significantly (P < 0.05) from their NAVEAL and hybrid counterparts. The changes in pH, as noted in the different protein matrices, might be due to the decrease in the available acid group as a result of the variations in the protein conformations during the application of pressure (McArdle, Marcos, Kerry, & Mullen, 2010).

The independent variables had no significant effect (P < 0.05) on the texture parameters of the plant-based patties. Contrastingly, HPP induced a linear increase in the hardness and chewiness of NAVEAL patties (Janardhanan et al., 2022). The varying effect of pressure might be due to the unfolding of the muscle fiber proteins at higher pressures (Gao, Zeng, Ma, Wang, & Pan, 2015). Similarly, the functionality of meat protein was reported to change after a threshold pressure of 200 MPa due to protein unfolding, agglomeration, aggregation, and network formation. The tenderization effect of HPP is absent at higher pressures

due to the aggregation of myosin and actin, leading to firmer meats (Bolumar et al., 2021). At the same time, the mean value hardness, gumminess, chewiness, and resilience of plant-based samples resembled NAVEAL.

3.2.2. Hybrid patties

The regression coefficient and other statistical results of the hybrid samples are presented in Table 3. The quadratic models (Table 3) could explain the curvature in the results for b^* , C^* and h^* values; the remaining color parameters were found to be non-significant. The a^* and h^* values of the hybrid patties were found to be significantly different (P < 0.05) from NAVEAL. The lower a^* value in the hybrid products might be due to the reduction in the myoglobin concentration caused by the presence of plant-based protein in the formulation. Unlike our results, a similar a^* value in HPP beef patties supplemented with 20% SPI compared to control meat patties was reported, which was attributed to the reduction of a^* value in the meat corresponding to the metmyoglobin formation by high pressure treatments (Bernasconi, Szerman, Vaudagna, & Speroni, 2020; McArdle et al., 2010).

Hardness, gumminess, and chewiness of the hybrid patties exhibited a linear relationship with the HPP pressure (P < 0.05). It could be observed that a rise in HPP pressure led to a hike in the parameters. Cohesiveness and adhesiveness were found to follow a significant quadratic relationship with HPP pressure (P < 0.05). The curvature in the data can be explained by this quadratic model. There was a significant linear effect of HPP pressure and HPP pressurization time on the resilience of the hybrid patties (Fig. 2c).

The hardness, gumminess and chewiness of hybrid samples were significantly higher than the plant based and NAVEAL samples (P < 0.05). Springiness and adhesiveness were found to be significantly different among the three formulations. The cohesiveness and resilience of hybrid patties were like the NAVEAL samples (P > 0.05).

The texture parameters of patties are related to the strength, number, and type of cross-links among the structural entities. The hardness is known to increase when the crosslinks become stronger (Zhou, Vu, Gong, & McClements, 2022). Varying results have been reported regarding the texture of meat batters added with plant proteins (Bernasconi et al., 2020; Danowska-Oziewicz, 2014). Moreover, the results varied based on the added protein, concentration, composition, and intrinsic characteristics (Gao, Zhang, & Zhou, 2015). The meat batters prepared with low gelling plant protein were harder and, reciprocally, high gelling softer. Non-meat proteins might act as water and fat binding agent, reducing the hardness (Youssef & Barbut, 2011). In the current study, the increase in texture attributes might be due to the interaction between the myofibrillar and plant proteins caused at higher pressures.

A significant (P < 0.05) linear effect of pressure and pressurization time was noted in the pH of the hybrid samples (Fig. 2d), which can be attributed to the redistribution of ions because of the changes in protein conformation (McArdle et al., 2010; Szerman, Ferrari, Sancho, & Vaudagna, 2019). The pH of the hybrid samples was significantly different from their meat and plant-based counterparts.

Most of the results for the HPP-treated hybrid patties conformed to the unique characteristics of the product, leaning neither towards the plant-based or NAVEAL patties.

Table 2

Prediction equations according to response surface methodology for physicochemical parameters of HPP treated plant-based samples.

Parameter	С	Р	t	P^2	t^2	P x t	R^{2} (%)
pH a* h*	6.3052 4.5800 1.6778	0.0004* 0.0036* -0.0004*	- 0.4810 -0.0595		- -0.0206* 0.0028		36.16 67.35 74.52

 $C = \text{constant}; P = \text{pressure}; t - \text{pressurization time}; R^2 = \text{determination coefficient}.$

* Significant terms in the model (P < 0.05), all the equations are averaged over blocks.



Fig. 2. Response surface plot for predicted color parameters in plant-based patties (a, b) and predicted resilience and pH in hybrid patties (c, d) according to the different pressure and time treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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rediction equations according to response surface methodology for physicochemical parameters of hybrid samples treated by HPP.	

Parameter	С	Р	t	P^2	ť ²	P x t	R ² (%)
pH	5.516	0.001	0.051	-	-	$-1.29 \ 10^{-4}$	57.21
b*	90.70	-0.28	-	$2.79 \ 10^{-4}$	-	_	51.57
C*	40.82	-	-3.43	-	0.17	_	39.29
h^*	2.446	-0.005	-	$5 \ 10^{-6}$	-	_	48.30
Hardness	13.530	0.031*	-	-	-	_	35.24
Gumminess	4.610	0.021*	-	-	-	_	40.96
Cohesiveness	0.057	0.002*	-	-2.10^{-7} *	-	_	58.36
Chewiness	2.270	0.018*	-	-	-	_	45.61
Resilience	0.1246	0.0001*	$1.83 \ 10^{-3}$ *	-	-	_	62.06
Adhesiveness	-0.061	-	-0.085	-	0.005*	-	45.91

 $C = \text{constant}; P = \text{pressure}; t - \text{pressurization time}; R^2 = \text{determination coefficient}.$

* Significant terms in the model (P < 0.05), all the equations are averaged over blocks.

3.3. Combined effects of HPP and SVCOOK on plant-based patties

3.3.1. Cooking loss, pH, and color

No significant effect of HPP pressure, HPP pressurization time or SVCOOK temperature was observed on the color parameters, pH or cooking loss of the plant-based patties. Similar non-significant effect of the combined treatment on the pH of NAVEAL patties was previously documented (Janardhanan et al., 2022).

The plant-based samples presented the lowest cooking loss, with the meat patties the highest (P < 0.05). Muscle fibers, when heat treated, denature, and shrinks, releasing the fluid trapped inside, whereas the plant-based raw material is a texturized vegetable protein (TVP) that has been denatured prior to the preparation of the patties; therefore, negligible cooking loss takes place. The minimal change in fluid holding capacity and microstructure changes might be the reason for the lowest cooking loss (Zhou et al., 2022).

The color parameters, except for the b^* value of plant-based patties, were significantly different (P < 0.05) from hybrid and NAVEAL patties. The plant-based patties reached the highest redness and yellowness with the lowest lightness values. C^* mean values of plant-based samples were similar (P > 0.05) to those of hybrid samples but different from the NAVEAL samples (P < 0.05). Minimum changes in the color values due to the heat treatment were observed in the plant-based patties, whereas heat attenuated metmyoglobin formation in meat patties and reduced it's a^* value. The smaller water globules and fat depots in meat reflect more light which does not occur in the plant-based samples, leading to the observed differences in lightness (Bakhsh et al., 2021; Barbut & Marangoni, 2019). Moreover, structural modifications in the myofibrillar packing and sarcoplasmic refraction due to the exertion of pressure might contribute to the observed L^* values (Hughes, Oiseth, Purslow, & Warner, 2014).

In similar studies with high-moisture meat analogs (HMMA)

containing commercial pea protein, lentil protein or faba bean protein mixed with pea isolates, wheat gluten and canola oil, the formulation significantly affected the yellowness and redness in these cooked HMMA patties (Kim, Miller, Laird, & Riaz, 2021). Bakhsh et al. (2021) found that TVP and texturized soy protein (TSP) isolate based patties decreased in lightness (L^*) and redness (a^*) after cooking, whereas raw beef patties were comparatively lighter and less red than the plant-based patties.

3.3.2. Texture

Pressure, pressurization time and SVCOOK temperature had a significant effect (P < 0.05) on springiness and hardness. The hardness and gumminess of the plant-based patties exhibited a linear trend with pressure. As the pressure increased from 350 to 600 MPa, a hike in hardness and gumminess was recorded. Springiness was found to follow a linear relationship with SVCOOK temperature (P < 0.05), while the rest of the terms in the model were found to be non-significant. With the rise in the SVCOOK temperature, the springiness of the patties increased. Parameters were significantly different (P < 0.05) from the hybrid and NAVEAL samples, except for adhesiveness, where no significant differences were observed. The hardness, springiness and chewiness of the plant-based patties were significantly lower than the NAVEAL and hybrid counterparts, which might be attributed to the lower cooking loss observed in the samples (Zhou et al., 2022).

Kim et al. (2021) reported that the formulation (proteins from pea, lentil and faba beans) in plant-based patties did not have any significant effect on hardness, whereas cohesiveness and gumminess were significantly affected by the formulation. Plant-based patties prepared with commercial TVP and TSP isolate have shown significantly lower hardness, chewiness, gumminess, cohesiveness and gumminess compared to control beef patties (Bakhsh et al., 2021).

3.4. Combined effect of HPP and SVCOOK on hybrid patties

The regression coefficients of the HPP and SVCOOK hybrid patties are presented in Table 4.

3.4.1. Cooking loss

HPP or SVCOOK did not significantly affect the cooking loss in the hybrid patties. Cooking loss mean values of hybrid patties were intermediate and significantly different (P < 0.05) from those recorded in NAVEAL and plant-based samples. An increase in the concentration of soy protein as a meat protein extender has decreased the cooking loss in beef patties (Cross, Berry, & Wells, 1980; Kassama, Ngadi, & Raghavan, 2003; Mansour & Khalil, 1997; Ray, Parrett, Stavern, & Ockerman, 1981; Troutt et al., 1992). Cooking loss of meat patties containing soy protein increases with a rise in cooking time and cooking temperature (Kassama et al., 2003). Meat releases fluid and melted fat under pressure and heat treatments. HPP induces dissociation and aggregation of proteins. As a result of the formation of aggregates, the surface area exposed

to water reduces, which in turn, increases cooking loss (Bernasconi et al., 2020). At the same time, the plant-based matrix has a lower cooking loss (as discussed in section *3.3.1*). The substitution of 50% meat protein with plant-based protein reduced the cooking loss. Improved, intermediate fluid holding capacity in the hybrid patties might be due to the formation of a loosely aggregated matrix between both the proteins, tending to be more like the characteristics of the plant-based samples. A lower cooking loss with a higher pulse substitution (yellow pea, chickpea, lentil at 25–75%) was reported in chicken patties, which concurs with our results (Chandler & McSweeney, 2022).

3.4.2. pH

No significant effect of HPP pressure, HPP pressurization time or SVCOOK temperature was detected on the pH of the hybrid patties. The pH value of the hybrid patties was intermediate and significantly different (P < 0.05) from those of the NAVEAL and plant-based patties. It could be noted that there was no considerable difference in the pH based on the processing variables. Comparable results were obtained by Ahmad, Rizawi, and Srivastava (2010) when soy protein isolate (25%) was added in buffalo meat sausage. According to Bell and Shelef (1978), minced beef mixed with 25% TSP has higher pH than patties with meat alone. Bakhsh et al. (2021) noted pH values higher than 6.5 in plant-based patties with methylcellulose.

3.4.3. Color

No significant effect of the processing parameters could be observed on the *L** and *a** values of the hybrid patties. HPP pressure, squared term of SVCOOK temperature and the interaction term HPP pressure x HPP pressurization time were found to have significant effect on the *b** value of the hybrid patties (P < 0.05). Yellowing was found to decrease progressively with increasing pressure, while the opposite was true for temperature (Fig. 3a–c).

The L* and h^* values of the hybrid samples were found to be significantly different (P < 0.05) from those of the NAVEAL and plantbased samples, whereas no significant difference was found in the b^* values and C^* . The mean of a^* values of hybrid samples did not differ from that of NAVEAL but was significantly lower (P < 0.05) than the plant-based samples. Redness of the hybrid patties resembled that of the NAVEAL-based counterparts. The light scattering properties of the hybrid samples were also comparable to the NAVEAL patties. In studies where an increased concentration of TSP was added in beef patties or sausages the L^* values significantly increased but the a^* value was not affected (Deliza, Saldivar, Germani, Benassi, 2002; Hidayat, Wea, & Andriati, 2017).

3.4.4. Texture

TPA parameters like hardness, cohesiveness, gumminess, chewiness, and resilience exhibited a significant quadratic relationship with SVCOOK temperature (Table 4). HPP pressurization time was also found to exert a significant linear effect on the cohesiveness of the hybrid

Table 4

Prediction equa	tions according to r	esponse surface meth	nodology for	physicochemica	l parameters of I	HPP and	SVCOOK treated	hybrid pat	ty samples.
	Ũ	.	0,	1 2	±				* *

Parameter	С	Р	t	Т	P^2	ť	T^2	Pxt	PxT	Txt	R ² (%)
<i>b</i> *	345	-0.0345	-1.624*	-10.300	-	-	0.0869*	0.00269		_	63.94
C*	364	-0.0349	-1.648*	-10.9600	-	-	0.0927*	0.00274*	-	-	65.41
h*	1.7509	-	-	-0.00718*	-	-	-	-	-	-	60.61
Hardness	-958	-	-	33.800	-	-	-0.282*	-	-	-	33.29
Gumminess	-678	-	-	23.7300	-	-	-0.1985*	-	-	-	41.33
Cohesiveness	-2.133	-	-0.0239	0.0954*	-	-	-0.000841*	-	-	0.000391*	63.65
Chewiness	-615	-	-	21.400	-	-	-0.1789*	-	-	-	48.27
Resilience	-0.869	-	-	0.0368	-	-	-0.000312*	-	-	-	31.35
Adhesiveness	1.54	-0.00492*	_	-0.0253*	-	-	-	-	0.000077*	-	63.86
Springiness	-1.998	0.001165	-	0.0842	-	-	-0.000626*	-	-0.000019*	-	48.72

 $C = \text{constant}; P = \text{pressure}; t - \text{pressurization time}; T = \text{SVCOOK temperature}; R^2 = \text{determination coefficient}.$

* Significant terms in the model (P < 0.05), all the equations are averaged over blocks.



Fig. 3. Response surface plot for predicted b* parameter (a, b, c) and selected texture parameters (d, e, f) in HPP + SVCOOK hybrid samples.

samples (Fig. 3e). HPP pressure and SVCOOK temperature and their interaction had significant effect on the adhesiveness and springiness of the hybrid patties (P < 0.05) (Fig. 3d and f). The lack of fit of the models were found to be non-significant (P > 0.05).

Means for hardness, gumminess, and chewiness of hybrid and NAVEAL patties were similar (P > 0.05), but significantly higher than plant-based patties (P < 0.05). No significant difference in the adhesiveness of the different formulations was observed while the springiness, cohesiveness and resilience differed among the samples, with

hybrid and NAVEAL samples exhibiting the maximum springiness and cohesiveness, respectively (P < 0.05).

Studies on meat patties prepared with soy flour protein and TSP found that cooking temperature significantly affected the toughness of patties extended with TSP (Kassama et al., 2003). No significant effect could be observed on the cohesiveness of the cooked patties prepared with soy flour protein (Heywood, Myers, Bailey, & Johnson, 2002). Simultaneously, an increase in the concentration of the soy flour protein in meat patties apparently reduced the hardness of the patties (Kassama

et al., 2003). Studies on addition of various concentrations of wheat bran in ground beef patties have reported a significant increase in hardness and gumminess with concomitant decrease in springiness and cohesiveness. Similarly, reduction in all texture parameter values were recorded with an increase in the amount of pulses added to chicken patties (Chandler & McSweeney, 2022). Yılmaz (2005) also reported an increase in firmness with increasing amounts of added bran, and comparable results were detected in meatballs prepared with oat bran (Yılmaz & Dağlıoğlu, 2003). Huang, Shiau, Liu, Chu, and Hwang (2005) reported a significant increase of gumminess when higher amounts of rice bran was added to Kung-wan, a ground and emulsified pork meatball.

3.5. Principal component analysis

The two main factors contributed to explain 75.67% of total variance, which suggested that two factors would be enough to reflect most of the overall characteristics of the patty samples. All color parameters as well as hardness, cohesiveness, adhesiveness, chewiness, gumminess, and resilience were associated to principal component 1 (PC1) (61.40% variance). Springiness, cooking loss and pH were associated to principal component 2 (PC2) (14.27% variance). The loadings of parameters for rotated factors are provided in Appendix Table A10. Fig. 4 displays the representation of factor scores for the HPP-SVCOOK samples. Three clusters were obtained, 75% of NAVEAL samples were located in the first quadrant, 100% of hybrid samples were placed in the second quadrant and 90% of plant-based samples were in the third quadrant. The hybrid patties showed an intermediate behavior in the physical parameters between the plant-based and the NAVEAL patties. The plant-based patties could be differentiated from the hybrid and NAVEAL patties using the PC1, since the plant-based patties clearly belonged to the negative range, whereas hybrid and most of the NAVEAL patties were in the positive range. The significant difference in the color parameters and most of the texture parameters as observed between plant-based patties compared to NAVEAL and hybrid patties separates them into different quadrants based on PC1. PC2 relates to distinguishing plant based and hybrid patties from NAVEAL patties, but the overall contribution of PC2 was only a small percentage. The significant difference in the cooking loss, springiness and pH of the NAVEAL patties compared to their counterparts is explained by the PC2.

4. Conclusion

The three types of patties were similar in protein and fat contents; only the moisture content of the NAVEAL patties was comparatively higher. Prediction equations for the physicochemical properties of the plant based and hybrid products were obtained using RSM. The HPPtreated plant-based patties had similar texture to NAVEAL patties, with varying results for the rest of the physicochemical properties. All HPP-treated patties were similar in L^* , C^* and cohesiveness. Except for cooking loss, pH, springiness, cohesiveness, L^* and h^* the HPP + SVCOOK hybrid patties had similar physical parameters (a^* , b^* , C^* , hardness, chewiness, gumminess, adhesiveness) to their NAVEAL counterparts, whereas the plant-based patties were not comparable in most physicochemical traits (i.e., cooking loss, pH, L^* , a^* , C^* , h^* , hardness, springiness, cohesiveness, chewiness, gumminess) to NAVEAL patties.

The enhanced color and texture properties due to the application of



Fig. 4. Plotting principal component for patties treated by combined HPP and SVCOOK (P = plant-based samples; H = hybrid samples; N = NAVEAL samples). Samples were coded as (pressure x pressurization time x SVCOOK temperature).

the dual (HPP + SVCOOK) technology could lead to innovative hybrid products with similar characteristics of the NAVEAL patties. The plantbased product could offer new on-the-counter options to vegan or vegetarian consumers but with the slightest similarity to its veal counterpart. Our findings can assist to narrow the gap in knowledge regarding the physicochemical attributes of alternative protein products subjected to HPP and (or) SVCOOK. Further research needs to be conducted to assess the microbiological, sensory, nutritional quality, shelf life, and consumer acceptability of the new products.

Funding

This project has received funding from the European Union's H2020 research and innovation program under the Marie Sklodowska-Curie grant (grant agreement No 801586).

CRediT authorship contribution statement

Rasmi Janardhanan: Investigation, Data curation, Formal analysis,

Writing – original draft. **Nelson Huerta-Leidenz:** Conceptualization, Supervision, Writing – review & editing. **Francisco C. Ibañez:** Formal analysis, Writing – review & editing. **Maria Jose Beriain:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

The data that has been used is confidential.

Acknowledgements

The authors extend sincere thanks to Dr. Fermin Mallor, Institute of Smart Cities, Universidad Pública de Navarra, Spain, for his guidance and help in preparing the statistics of the experimental designs. Open access funding provided by Universidad Pública de Navarra.

Appendix

Table A1

Central composite experimental design matrix for HPP treatment for NAVEAL, plant-based and hybrid patties.

Run	Block	Pressure (MPa)	Pressurization time (min.)
1	1	475	15
2	1	600	10
3	1	475	10
4	1	475	5
5	1	350	10
6	1	475	10
7	1	475	10
8	1	600	5
9	1	350	5
10	1	600	15
11	1	475	10
12	1	350	15
13	1	475	10

 Table A2

 Central composite experimental design matrix for HPP + SVCOOK treatments of plant-based patties.

Run	Block	Pressure (MPa)	Pressurization time (min.)	SVCOOK temperature (°C)
1	1	350	5	55
2	1	350	5	65
3	1	350	10	60
4	1	350	15	65
5	1	350	15	55
6	1	475	5	60
7	1	475	10	60
8	1	475	10	60
9	1	475	10	60
10	1	475	10	60
11	1	475	10	55
12	1	475	10	65
13	1	475	10	60
14	1	475	10	60
15	1	475	15	60
16	1	600	5	65
17	1	600	5	55
18	1	600	10	60
19	1	600	15	55
20	1	600	15	65

Table A3

Central composite experimental design matrix for HPP + SVCOOK treatments of NAVEAL and hybrid patties.

Run	Block	Pressure (MPa)	Pressurization time (min.)	SVCOOK temperature (°C)
1	3	475	10	60
2	3	475	15	60
3	3	600	10	60
4	3	475	10	55
5	3	475	5	60
6	3	350	10	60
7	3	475	10	65
8	3	475	10	60
9	1	475	10	60
10	1	600	5	65
11	1	350	5	55
12	1	600	15	55
13	1	350	15	65
14	1	475	10	60
15	2	600	15	65
16	2	600	5	55
17	2	350	5	65
18	2	350	15	55
19	2	475	10	60
20	2	475	10	60

Table A4

Physicochemical parameters of HPP treated samples based on their formulation.

Parameter	Plant-based		Hybrid		NAVEAL	
	Mean	SD	Mean	SD	Mean	SD
рН	6.49 ^a	0.06	6.07 ^b	0.8	5.74 ^c	0.04
L^*	51.93 ^a	1.78	52.21 ^a	2.26	51.58 ^a	3.56
a*	8.78^{b}	0.59	8.16^{b}	1.78	10.90^{a}	1.54
<i>b</i> *	23.67 ^a	3.37	24.07^{b}	3.47	22.61 ^a	2.05
<i>C</i> *	25.28 ^a	3.08	25.47 ^a	3.52	25.16 ^a	1.84
h*	1.21^{a}	0.06	1.24 ^a	0.06	1.12^{b}	0.07
Hardness	15.51 ^b	3.82	28.44 ^a	4.68	13.94 ^b	3.41
Springiness	$0.80^{\rm b}$	0.01	0.75 ^a	0.03	0.71 ^c	0.02
Cohesiveness	0.37 ^b	0.05	0.51 ^a	0.03	0.48 ^a	0.03
Gumminess	6.17 ^b	2.00	14.48 ^a	2.87	6.68 ^b	1.79
Chewiness	4.97 ^b	1.63	10.83^{a}	2.36	4.70 ^b	1.24
Resilience	0.18^{a}	0.02	0.19^{a}	0.01	0.18^{a}	0.01
Adhesiveness	-0.04^{a}	0.03	-0.35^{b}	0.12	-0.69^{c}	0.14

Mean values with different superscript letters in the same row are different (P < 0.05) observed between the groups in Tukey's pairwise comparison between their formulations (meat, plant based and hybrid samples).

Table A5

 $Physicochemical \ parameters \ of \ HPP + SVCOOK \ treated \ samples \ based \ on \ formulation.$

Parameter	Plant-based		Hybrid		NAVEAL	
	Mean	SD	Mean	SD	Mean	SD
рН	6.39 ^a	0.04	6.08 ^b	0.05	5.80 ^c	0.04
Cooking loss (%)	0.99 ^c	0.92	4.93 ^b	0.93	25.06 ^a	3.71
L^*	52.28 ^c	1.83	55.23 ^b	2.00	57.18 ^a	3.18
a*	8.34 ^a	0.45	5.40 ^b	0.98	6.12 ^b	1.40
b*	22.33 ^a	3.19	21.12 ^a	2.70	20.79 ^a	2.15
C*	23.86 ^a	3.09	21.82 ^{ab}	2.78	21.69 ^b	2.38
h*	1.21 ^c	0.04	1.32 ^a	0.03	1.29 ^b	0.04
Hardness	21.96 ^b	4.46	51.65 ^a	6.27	49.64 ^a	8.56
Springiness	0.77 ^b	0.04	0.81 ^a	0.02	0.68 ^c	0.04
Cohesiveness	0.33 ^c	0.06	0.55^{b}	0.02	0.60 ^a	0.04
Gumminess	7.78 ^b	2.68	28.46 ^a	4.00	29.80 ^a	6.36
Chewiness	6.01 ^b	2.10	23.15 ^a	3.32	20.59 ^a	4.96
Resilience	0.14 ^c	0.02	0.21 ^b	0.01	0.23 ^a	0.02
Adhesiveness	-0.34^{a}	0.73	-0.12^{a}	0.07	-0.10^{a}	0.10

Superscript letters indicate the significant difference in each row (P < 0.05) observed between the groups in Tukey's pairwise comparison between their formulations (meat, plant based and hybrid samples).

Table A6

Parameter contributions for rotated principal components, based on loadings.

Parameter	Principal component 1	Principal component 2
Chewiness	0.960	0.110
Hardness	0.930	0.192
Gumminess	0.922	0.289
Cohesiveness	0.884	0.407
Resilience	0.860	0.450
L^*	0.573	0.437
Adhesiveness	0.519	-0.056
b^*	-0.343	-0.140
<i>a</i> *	-0.834	-0.062
Cooking loss (%)	0.417	0.861
pH	-0.675	-0.696
Springiness	0.193	-0.949

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