agriculture

MDPI

Article

The Effect of Protein Derivatives and Starch Addition on Some Quality Characteristics of Beef Emulsions and Gels

Daniela Ianiţchi [1], Livia Pătraşcu [2], Floricel Cercel [3], Nela Dragomir [1], Iulian Vlad [1] and Marius Maftei [1,*]

[1] Department of Production and Processing Technologies, Faculty of Animal Productions Engineering and Management, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464 Bucharest, Romania; daniela.ianitchi@usamv.ro (D.I.); nela.dragomir@usamv.ro (N.D.); iulian.vlad@usamv.ro (I.V.)

[2] Cross-Border Faculty, Dunarea de Jos University of Galati, 111 Domneasca Str., 800201 Galati, Romania; livia.patrascu@ugal.ro

[3] Department of Food Science, Food Engineering, Biotechnologies and Aquaculture, Faculty of Food Science and Engineering, Dunărea de Jos University of Galati, 111 Domneasca Str., 800201 Galati, Romania; floricel.cercel@ugal.ro

* Correspondence: marius.maftei@usamv.ro; Tel.: +40-0742-004-900

Abstract: Starch and plant or animal proteins represent a rich source that can be used for fortifying meat products. The present study aimed to analyze how the different additives used (soy protein isolate, chickpea flour, lupine concentrate, sodium caseinate and starch, in 2% concentration) influence the rheological properties of beef emulsions and gels, cooking losses and the texture of the finished products. Rheological parameters G′ (storage modulus), G* (complex modulus) and Delta angle were determined by increasing the temperature from 5 to 70 °C, with a rate of 1 °C/min. The study highlighted that the addition of vegetable proteins (soy, chickpeas and lupine) improved strength of meat gels (G′ > 1057.8 Pa), while the addition of sodium caseinate and starch reduced the consistency of the gel network structure (G′ < 1057.8 Pa). All additions led to a decrease in heat treatment losses (a reduction of maximum 62% of cooking loss, from 11.89% for control to 4.54% in case of samples with added starch) and the hardness of heat-treated products. The maximum reduction of hardness was observed for samples with added starch, from 2.83 kgf to 1.08 kgf.

Keywords: meat formulations; beef; rheological properties; plant-based ingredients

Citation: Ianiţchi, D.; Pătraşcu, L.; Cercel, F.; Dragomir, N.; Vlad, I.; Maftei, M. The Effect of Protein Derivatives and Starch Addition on Some Quality Characteristics of Beef Emulsions and Gels. *Agriculture* **2023**, *13*, 772. https://doi.org/10.3390/agriculture13040772

Academic Editors: Gabriela Maria Grigioni, Anibal Pordomingo, Ignacio Arturo Domínguez Vara and Ernesto Morales Almaráz

Received: 20 February 2023
Revised: 23 March 2023
Accepted: 24 March 2023
Published: 27 March 2023

1. Introduction

Obtaining meat products has lately involved experimenting with different formulas that include products/ingredients of vegetable or animal origin in order to improve the quality of finished products, to supplement the protein intake through cheaper sources or increase yields in finished products. In 1970, it was mentioned the addition of soy derivatives to meat preparations for their ability to emulsify fats, stabilize emulsions, improved nutritional properties and yields [1].

The quality of the finished products depends on the raw materials quality, the ingredients used and the processing technologies.

Minced meats are considered emulsions whose stability depends on the quantity and quality of proteins that influence the binding of fats, water retention, generating gels that can be filled, complex or mixed [2]. Gel formation is a combination of intrinsic protein factors (hydrophobicity, electrostatic interactions, disulphide bonds, molecular weight, amino acid composition) and extrinsic factors (protein concentration, pH, temperature, ionic strength, pressure) that control the degree of unfolding or denaturation and refolding or association in the ordered protein matrix [3,4].

Amylaceous or protein derivatives (of vegetable or animal origin) have good emulsifying properties favoring the formation of gels; they have the ability to bind water, reduce heat

treatment losses and influence the sensory properties of finished products, thus dictating the degree of acceptance [5,6].

Soy proteins help meat emulsions stabilization, forming gels that act as a matrix for water and fat retention [7–9]. The study of replacing the fat in beef compositions with soy protein, whey protein or gluten showed that soy proteins are more effective in retaining water and reducing boiling loss than whey and gluten proteins [7]. Urbonaite et al. (2015) studied the quality of soy protein gels and showed that there was a correlation between the fineness of soy gels and their ability to retain water, coarse gels losing water more easily [10]. Pork gels in which soy derivatives were added showed different properties of fineness/porosity, cohesiveness and breaking strength, depending on the treatments previously applied to the derivatives (native, heated, hydrolyzed) [11]. The association of soy protein isolate with carrageenan (soy protein isolate and carrageenan mixture) did not generate hardness increases in low-fat pork batters compared to soy-only samples but decreased heat treatment loss [12]. The incorporation of textured soy granules in beef burger patties significantly reduced the color, appearance, flavor, tenderness, juiciness, taste and overall acceptability in comparison with control but significantly improved cooking loss [13]. Das et al. (2007) analyzed the influence of adding full fat soy paste to goat meat patties and found that emulsion stability and sensory attributes of patties decreased with increasing levels of full fat soy paste [14].

The addition of polysaccharides in food is used for gelling, stabilization and improvement of texture, fat emulsification, flavor retention, and syneresis control [15]. Modified starch is a potentially good source for controlling the binding properties of frankfurter products. Analyzing the emulsifying capacity of meat compositions with the starch addition, it was found that acid-modified starches had a higher emulsifying capacity and imprinted higher hardness to heat-treated products, compared to native potato starch and dextrinized starch [16]. The increase of starch concentration in the meat pastes generates the decrease of the emulsification capacity and hardness of the samples. Added starch in mechanically deboned turkey meat led to a decrease in heat treatment losses, an increase in juiciness, but also a reduction in flavor intensity [9].

There are numerous studies on the use of casein in meat pastes to improve the quality of emulsions and gels [9,17–21]. Colmenero et al. (2010) studied the stabilization with sodium caseinate of the emulsions for Frankfurters in which pork fat was replaced with olive oil, the obtained products having good water and fat binding properties, with stronger structures, but comparable general acceptability scores to the Frankfurters formulated with pork fat [17]. The texture of chicken sausages was improved by added casein, soybean protein and whey protein isolate, modified by transglutaminase, the addition of these biopolymers being able to allow reduction in phosphate content without loss in texture [18]. The use of casein-based hydrogels was studied for the controlled delivery and release of biomolecules such as vitamins and unsaturated fatty acids [20], for the addition of fish oil to meat products and the development of functional foods [19].

Legume proteins (peas, lentils, lupine, chickpeas, etc.) were increasingly used among meat analog producers [5,22]. They are of particular interest due to their nutritional value, availability, low price and health benefits [23,24].

Lupine is a legume with high protein content, balanced in terms of amino acid content, low in alkaloids and high in carbohydrates and insoluble fiber, which allows its use to replace animal proteins [25]. Studying the functional properties of lupine, Lara-Rivera et al. (2017) showed that the indexes of emulsion activity and emulsion stability were influenced by the pH, different varieties influencing water-holding capacity, but not oil holding capacity [26]. When the fat was replaced in sausage pasta with inulin or lupin-kernel fiber products were obtained that have provided a higher degree of satiety to subjects and a good degree of acceptability [27]. Development of low-fat meat products by adding protein or carbohydrate derivatives was also studied by other researchers [8,28].

The utilization of chickpea in different forms (roasted, fermented, baked, extruded) for the preparation of various food products, such as hummus, muffin, biscuits, pasta, ready to

eat snacks, pork bologna, chicken nuggets has been studied [29]. The addition of chickpea protein concentrates to obtain "Merguez" sausage had a significant impact on the textural properties, improved color stability and decreased lipid oxidation [30]. Chickpea protein isolates produced by isoelectric precipitation had higher solubility, formed emulsions with smaller droplet size, and showed high emulsifying activity and stability that were comparable to the soy protein isolates [24]. Studies showed that most of the functional properties of chickpea proteins were comparable with or superior to those of soy and animal origin proteins tested [31]. Some researchers mention the increased cooking yield, cohesiveness and firmness of low-fat pork bologna in whose composition was added chickpea flour [32].

Most of the rheological studies refer to simple chemical systems such as protein concentrates, protein extracts, polysaccharide solutions, pectic gels, etc. The current study aimed to investigate the gelling behavior at high temperatures for meat compositions as used in industry, compositions that are complex systems made up of meat proteins, fats, water, salt, nitrites, polyphosphates, to which starch or protein derivatives are added. These models are found in practice when obtaining minced meat products (salami, sausages) or canned minced meat. The rheological behavior of the proposed formulations is the result of the interactions between the system components and could allow the establishment of correlations with the yield, hardness and juiciness of cooked products and acceptance by consumers.

On the other hand, such studies allow the creation of new products by valuing vegetable or animal derivatives, which are more accessible, and ensure the development of a sustainable food industry.

2. Materials and Methods

2.1. Raw Materials

Emulsions from beef, fat, water, salt, sodium nitrite and polyphosphates were used as basic emulsions for different studied formulations. Beef, purchased from SC Intern SRL, Romania, had the following chemical composition: 74.15% water, 20.12% proteins, 4.56% lipids and 0.93% ash. The chemical composition were assessed using the following methods: the moisture content with a drying oven SLW 53 (Pol-Eko-Aparatura, Wodzisław Śląski, Poland)—AOAC 39.1.02; the protein content, Kjeldahl method (Raypa Trade, R. Espinar, S.L., Barcelona, Spain)—AOAC 39.1.15; the fat content, Soxhlet method (Gerhardt GmbH & Co.KG, Königswinter, Germany)—AOAC 39.1.08 and the ash content with calcination oven LAC s.r.o. (Židlochovice, Czech Republic)—AOAC 39.1.09 [33].

The control sample—beef-based emulsion (B), consisted of 70% muscle tissue (*Semimembranosus*), 30% fatty tissue (from SC Intern SRL, Ilfov, Romania), 15% water, 2.0% salt, 0.5% polyphosphates, and 0.015% sodium nitrite. To this basic emulsion, 2% of soy protein isolate (BSPI) (Supro 33), containing 90% protein, 6% water, 1.5% fat, and 5% ash (according to manufacturer), chickpea flour (BCh) (wih 20.8% protein, 6.24% fat, 9.5% water), lupine concentrate (BL) (Lupipro 550), containing 75% protein, 0,5% fat, and 2.45% ash, sodium caseinate (BCs) (RovitaHV2) with 88.0% protein, 8% water, 1.5% fat, 6% ash, and native potato starch (BSt) (0.05% protein, 0.09% fat, 0.5% ash, 21% water) were added, to which hydrating water was also added. The protein derivatives, starch, salt, nitrites and polyphosphates were purchased from SC Aromatique Food Ltd., Romania. Hydrating water was added according to the recipes used in production, as presented in Table 1.

The meat, fat, chilled water, auxiliaries and protein and starch additions were finely ground at 3000 rpm for 40 s, with Blixer 3 (Robot Coupe, Montceau en Bourgogne, France). The meat emulsions were stored for 12 h at 0–4 °C until rheological testing and cooking. The filling of the composition into membranes was performed under laboratory conditions with an EKM3710 robot (Electrolux, Stockholm, Sweden), using synthetic membranes (Pioneer RTOR, Iaşi, Romania) with a diameter of 20 mm, the samples being then heat treated in hermetically sealed containers, at a temperature of 70 °C, at a growth rate of

1 °C/min, for 10 min in the thermal center of the product, measured with a thermocouple. The cooled samples were stored at 0–4 °C for 12 h prior to investigations.

Table 1. Formation of beef emulsions fortified with different protein and amylaceous derivatives.

Ingredients, g	Samples					
	B	**BSPI**	**BCh**	**BL**	**BCs**	**BSt**
Beef	70	70	70	70	70	70
Fat	30	30	30	30	30	30
Water	20	28	28	28	28	28
NaCl	2	2	2	2	2	2
Polyphosphates	0.3	0.3	0.3	0.3	0.3	0.3
$NaNO_2$	0.015	0.015	0.015	0.015	0.015	0.015
Chickpea flour	-	-	2	-	-	-
Lupine protein concentrate	-	-	-	2	-	-
Soy protein isolate	-	2	-	-	-	-
Sodium caseinate	-	-	-	-	2	-
Starch	-	-	-	-	-	2

B—control sample (no additions); BCh—beef emulsion with chickpea flour; BL—beef emulsion with lupine concentrate; BSPI—beef emulsion with soy protein isolate; BCs—beef emulsion with sodium caseinate; BSt—beef emulsion with starch.

2.2. Rheological Properties Determinations

Rheological properties of meat emulsions were studied by the means of low amplitude oscillatory tests using a TA2000ex Rheometer (TA Intruments Ltd., New Castle, DE, USA). The temperature was controlled with a Peltier Plate. Samples were placed between Petier Plate and a 2° cone geometry of 40 mm in diameter. The gap between plates was set to 2 mm. Water evaporation during tests was avoided by covering the edges with a silicone oil (density = 0.98–1.01 g/mL, Merck KGaA, Darmstadt, Germany). Temperature ramp tests were performed by increasing the temperature from 5 to 70 °C, with a rate of 1 °C/min. During tests, strain value was set at 0.5% determined to be within linear viscoelastic range with a strain sweep test. The oscillatory frequency was set at 1 Hz. Rheological parameters G′ (storage modulus), G″ (loss modulus), G* (complex modulus) and Delta angle were recorded. Protein denaturation temperature was assessed by calculating the first derivative of the third-order polynomial equation that best fitted the inflexion domains (the highest R2 registered), namely 20–70 °C [34,35].

2.3. Textural Analysis

The cutting force, also known as Warner-Bratzler shear force was determined using the TA-XT Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were: 25 kg cell, cutting speed 1.5 mm/s, piston retraction speed 10 mms^{-1}, distance 30 mm. The maximum force required to cut the sample was recorded.

2.4. Cooking Losses

Samples were weighed before and after the heat treatment. The total losses, expressed in water and fat, were calculated with Equation (1).

$$\text{Cooking loss } [\%] = 100\frac{M1 - M2}{M1} \quad (1)$$

where: $M1$—sample weight before the heat treatment; $M2$—sample weight after heat treatment.

2.5. Microscopic Investigations

The appreciation of gel structures after heat treatment of the samples was highlighted by the means of images obtained with a Stereomicroscope with integrated digital camera, type Leica E.Z.4, zoom magnification 8X (Leica Microsystems, Wetzlar, Germania).

2.6. Statistical Analysis

The statistical analysis of the results was carried out using Statgraphics Centurion XVI.I software. The data were subjected to single factor ANOVA analysis, considering a significance level of 95.0%. Fisher's least significant difference (LSD) test at a 95.0% confidence level was used to determine differences between mean values. All analyses were carried out in duplicate for rheological properties and five replicas for the rest of the investigations, and data were reported as mean values ± standard deviation.

3. Results and Discussion

3.1. Rheological Properties

In order to estimate the effect of the thermal treatment on the rheological behavior of the meat butters fortified with different protein and amylaceous derivatives, the viscoelastic properties of the emulsions were studied by performing temperature ramp tests. The rheological transformations of the beef compositions with protein and amylaceous additions induced by heat are presented graphically in Figure 1, as G* (complex modulus) evolution as a function of temperature. Complex modulus is a measure of viscoelasticity including both G′ and G″ moduli: $G^* = \sqrt{G'^2 + G''^2}$.

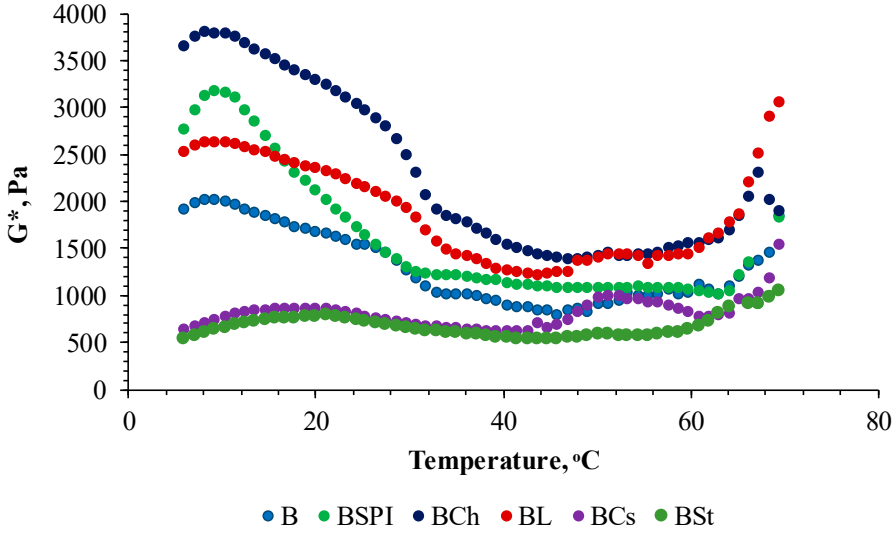

Figure 1. The effect of different non-meat ingredients on rheological behavior of beef emulsions during small amplitude oscillatory temperature ramp test. B—control sample (no additions); BCh—beef emulsion with chickpea flour; BL—beef emulsion with lupine concentrate; BSPI—beef emulsion with soy protein isolate; BCs—beef emulsion with sodium caseinate; BSt—beef emulsion with starch.

As a general trend, it could be observed that over the considered temperature range, the G′ values were significantly higher than G″ ($p > 0.05$) for all samples, indicating a predominant elastic behavior, with a solid like structure (data not shown).

As can be seen from Figure 1, during the first phase of the heating process, at low temperature (5–30 °C), G* values decreased. The phenomenon could be associated with some combined chemical transformations such as fat melting, thermal protein weakening, polypeptide chains unfolding and destruction of the proteins structure, and partial dehydration of proteins. At the same time, the water removed from meat particles during

the process, becomes free water and acts as a lubricant. In the range of 30–35 °C, it was estimated that the low elasticity of the meat emulsion was due to the fact that myofibrillar proteins (actin, actomyosin and myosin) undergo major structural and conformational changes during heating that cause their denaturation. Tropomyosin also separates from actin filaments, and above 40 °C the endothermic transition of the myosin molecule and its subfragments, HMM and LMM, takes place [36].

Above the temperature of 45 °C, all rheograms presented an ascending evolution due to the formation of new, more stable bonds between the polypeptide chains and the coagulation of the structural proteins that determine the formation of the gel structure.

Protein denaturation and gel formation is usually marked by the presence of inflection points in the G* curve. As can be seen from Figure 1 all samples presented G* increasing values approximately after 40 °C, in accordance with other findings [35,37]. As a general trend, the rheograms obtained for all samples showed the same profile in the temperature range 45–70 °C, major differences being found in the range of 5–45 °C. In case of control, the G* curve presented a continuous increase after 40 °C up to the end of the test. In this respect, it can be mentioned that the main contractile muscle proteins, myosin and actin, present denaturation points between 54 and 58 °C in case of myosin and 80–83 °C for actin, while for collagen and other sarcoplasmic proteins 65 to 67 °C were reported [38].

The increase of G* value may be due to the cross-links formed between meat proteins, meat proteins and other constituents of the compositions, which make the emulsions more cohesive, less sensitive to temperature variation and which subsequently develop gel network during heating.

The much higher values of G* in the samples with vegetable protein additions at the beginning of the test, compared to the control sample, could be the result of some chemical interactions between meat and vegetable proteins (soy, chickpeas and lupine), vegetable proteins, proteins and water, and also proteins and fat. The same trend can be observed for G′ and delta values until denaturation temperature, as shown in Table 2.

Table 2. Some rheological parameters shown by beef emulsions supplemented with different non-meat ingredients.

Sample	Denaturation Temperature (D.T.)	Delta Value, °		G′ Value, Pa	
		5 °C—D.T.	D.T.—70 °C	5 °C—D.T.	D.T.—70 °C
B	47.9 ± 0.23 ^{b}	16.39 ± 0.21 ^{a}	18.22 ± 0.10 ^{a}	1353.4 ± 9.8 ^{a}	1057.8 ± 8.6 ^{c}
BSPI	46.7 ± 0.26 ^{b}	18.18 ± 0.25 ^{b}	16.12 ± 0.47 ^{b}	1732.8 ± 2.7 ^{b}	1068.0 ± 3.7 ^{c}
BCh	50.3 ± 0.34 ^{b}	16.74 ± 0.30 ^{a}	15.50 ± 0.20 ^{b}	2496.4 ± 40.5 ^{c}	1606.2 ± 20.2 ^{d}
BL	47.4 ± 0.75 ^{b}	16.75 ± 0.19 ^{a}	15.86 ± 0.11 ^{b}	1883.1 ± 23.7 ^{d}	1693.4 ± 42.1 ^{d}
BCs	42.6 ± 0.16 ^{a}	30.13 ± 0.37 ^{c}	17.78 ± 0.34 ^{a}	646.4 ± 6.4 ^{e}	864.0 ± 24.1 ^{b}
BSt	42.6 ± 0.88 ^{a}	31.63 ± 0.03 ^{d}	17.98 ± 0.35 ^{a}	571.4 ± 6.8 ^{e}	636.6 ± 16.1 ^{a}

Values with similar letters within a column are statistically similar at a 95.0% confidence level. B—control sample (no additions); BCh—beef emulsion with chickpea flour; BL—beef emulsion with lupine concentrate; BSPI—beef emulsion with soy protein isolate; BCs—beef emulsion with sodium caseinate; BSt—beef emulsion with starch.

These may cause the formation of dense and elastic emulsions, which includes the water and fat, while in the control sample only solubilized meat proteins bind water and fat, resulting in a less dense, adhesive and elastic composition. Additionally, fibers contained by vegetable ingredients have a high hydration capacity and can favor the elasticity of the mixture.

At the transition temperature of the protein sol to gel, the structure of the gel network was strongly consolidated by continuing the heat treatment. Heating of the samples above the temperature of 50 °C determined an increase in the elasticity of the gels formed, an increase resulting from the fact that the partially or totally denatured proteins form new bonds, more stable, with the generation of a three-dimensional network.

Table 2 present temperature values associated with the beginning of protein denaturation and gel formation, as determined from G' vs. temperature curves by first derivative of the third-order polynomial equation. The third order polynomial equation was applied for G' values between 20 and 70 °C. It could be seen that for samples containing different legume derivatives (soy protein isolate, chickpea flour and lupine protein concentrate) temperature values associated with the beginning of the denaturation process and gel formation were rather close to the control sample, while when caseinate and starch were used, temperature values associated with the phenomenon were much lower. The same observation was made also in the case of storage modulus values. Average Delta and G' values were calculated before and after the estimated denaturation temperature (Table 2). Usually, G' values are associated with material's consistency. Thus, it could be appreciated that both row emulsions and thermal gels containing caseinate and starch presented a lower consistency in comparison to samples supplemented with protein derivatives from legumes.

Samples with starch and sodium caseinate (Figure 1) showed much lower G* values compared to the control sample, but very close to each other at the first part of the test (Table 2). The reduction in the modulus in the case of starch and caseinate compositions can be explained by the high hydration ratio. This resulted in an increase in the free water in the system which would reduce the interactions between the particle surfaces and the viscosity of the liquid phase [39].

The loss modulus had a general downward evolution throughout heating with some small deviations. Thus, the control samples, with starch and sodium caseinate, recorded slightly increasing evolutions on the interval 5–10 °C and 5–15 °C, followed by descending evolution.

At the end of the test, loss modulus (G") presented lower values for the samples with protein of chickpeas, soy, lupine, control and slightly higher for sodium caseinate and starch. Temperatures of 60–70°C also corresponded to the gelling temperatures of plant proteins.

3.2. The Effect of Protein and Starch Addition on the Cutting Force

The influence of protein derivatives and starch addition on the texture of the products was appreciated through cutting force (Table 3). It can be observed that the highest cutting force was recorded for the control sample, followed by the samples with protein additions. The samples with the highest cutting forces were those with added soy and lupine, followed by the sample with chickpea flour addition. However, statistical similarities resulted for lupine and chickpea containing samples, the one with soy protein isolate in composition being significantly harder than the previous two. The lowest shear forces were recorded for samples containing sodium caseinate and starch, the former being though the softest ($p < 0.05$). Thus, results are in agreement with rheological behavior. The decrease in the hardness of products obtained from different types of meat to which vegetable protein derivatives or starch have been added has been reported by various researchers [40–43].

Table 3. Variation of some textural parameters depending on the nature of the additives used.

Parameter	Composition					
	B	**BSPI**	**BCh**	**BL**	**BCs**	**BSt**
Cutting force, kgf	2.83 ± 0.038 ^{e}	2.65 ± 0.023 ^{c}	2.13 ± 0.043 ^{d}	2.52 ± 0.013 ^{d}	1.65 ± 0.006 ^{b}	1.08 ± 0.002 ^{a}
Cooking losses, %	11.89 ± 1.138 ^{d}	9.07 ± 0.115 ^{c}	7.69 ± 0.163 ^{b}	8.64 ± 0.143 ^{c}	5.31 ± 0.152 ^{a}	4.54 ± 0.188 ^{a}

Values with similar letters within a row are statistically similar at a 95.0% confidence level. B—control sample (no additions); BCh—beef emulsion with chickpea flour; BL—beef emulsion with lupine concentrate; BSPI—beef emulsion with soy protein isolate; BCs—beef emulsion with sodium caseinate; BSt—beef emulsion with starch.

It can therefore be said that samples that showed the lowest resistance to chewing, hence that formed the weakest gel network, were those containing sodium caseinate and

starch, followed by the products that incorporated vegetable proteins, then the control sample, which also showed the strongest structure after heat treatment.

The cutting force is influenced by a number of factors such as the type of meat, the ratio of muscle tissue-connective tissue-fatty tissue in the compositions, the water content of the compositions, the degree of chopping of the components, the boiling temperature but also the additives used to make the compositions. In comparison, for whole muscles, textural parameters are mostly influenced by the tumbling regime and the brine injection level [44], the salts or enzymatic preparations addition [45].

Additionally, the shear force can be correlated with the functional properties of the additives used, more precisely with their ability to bind water. Thus, the components able to bind and incorporate in the structure the largest amount of water, leading to the softest textures.

3.3. The Influence of Amylaceous and Protein Additives on Cooking Losses

The aqueous phase of minced and emulsified meat paste is a complex mixture of muscle fibers, myofibrils, actin, myosin, actomyosin and other myofibrillar proteins, as well as sarcoplasmic and stromal proteins that occur either in dissolved state or in the form of aggregates. As a result of the applied heat treatment, meat proteins and proteins from other sources initially suffer a denaturation phenomenon and then participate in various protein–protein interactions that contribute to the formation of the gel network structure which sequester water and stabilize the emulsion. As can be seen from Table 3, increasing the concentration of proteins in the aqueous phase of the emulsions, contributed to improving the water retention capacity of the system and to stabilizing the emulsions, reducing the total losses during heat treatment (water + fat) in relation to the control sample and increasing the yield of the finished product. The same observation could be made in the case of starch addition. The increase in salts in addition to meat compositions also generated a decrease in the cooking loss [46].

In the case of vegetable protein additions, the lowest loss was recorded in the sample with chickpea flour addition, with statistically different values ($p < 0.05$) in comparison to sample containing soy protein isolate and lupine, a situation that can be explained due to the fiber content that was also capable of retaining water. Moreover, with exception of the sample containing chickpea flour, there was observed a linear correlation ($R^2 = 0.8878$) between denaturation temperature (Table 2) and cooking losses.

The lowest losses were recorded in the samples with sodium caseinate and starch additions. These results were also correlated with the textural properties; the lower values recorded for the shear force indicating a softer structure for the starch and caseinate compositions, most likely due to the water retained in the gel network and the type of bonds formed. The obtained results were consistent with those published by other researchers. The use of cereal (oat, wheat) and legume flours (soy, chickpea) instead of fat significantly increased the moisture and fat retention of cooked meat patties [47]. For beef sausages, in which part of the meat was replaced with texturized vegetable protein, the hydration capacity increased [40]. Other researchers appreciated that starch addition generates high water-oil retention capacity in the system and improves rheological properties and stability of meat emulsions [48].

A reduction in heat treatment and refrigeration losses was also recorded, correlated with a decrease in the product hardness for the starch samples and an increase in the yield after boiling for the protein-added compositions compared to the control. Other studies showed that the addition of starch improved product juiciness [49,50].

3.4. Investigation of the Microscopic Structure of Beef Compositions with Different Additions, Subjected to Heat Treatment

The section appearance of meat products emulsified and restructured by heat treatment is one of the sensory parameters that define the quality of meat preparations.

From Figure 2a–f it can be seen that the structure of the samples in section was different in terms of fineness of composition and the presence and frequency of gel-filled gaps. There were also obvious differences in the color of the products, another criterion that defines the sensory quality of meat dishes.

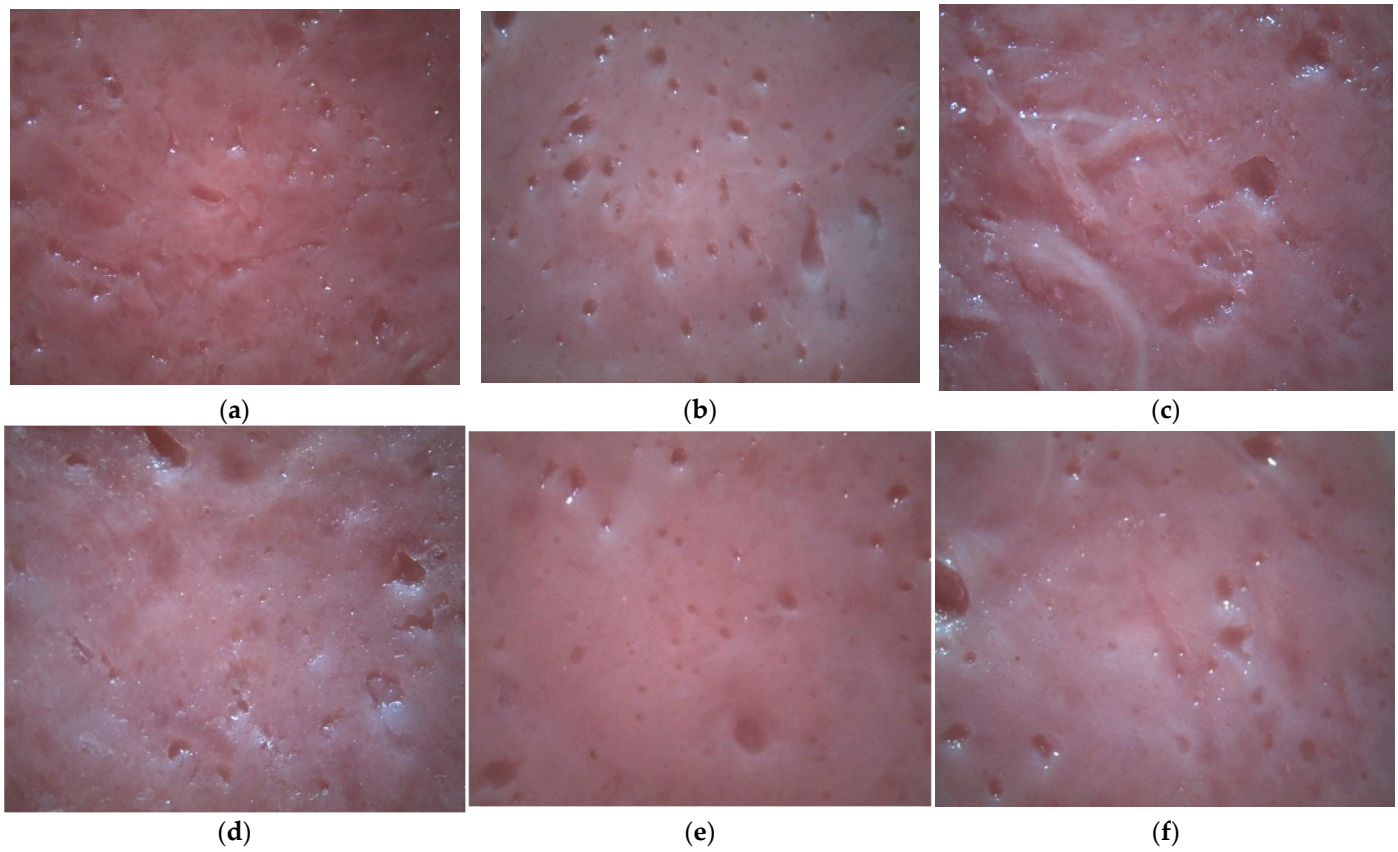

Figure 2. Stereomicroscopic image of beef compositions with different additions. B—control sample (no additions); BCh—beef emulsion with chickpea flour; BL—beef emulsion with lupine concentrate; BSPI—beef emulsion with soy protein isolate; BCs—beef emulsion with sodium caseinate; BSt—beef emulsion with starch. (**a**) Stereomicroscopic image of the B gel; (**b**) Stereomicroscopic image of the BCh gel; (**c**) Stereomicroscopic image of the BL gel; (**d**) Stereomicroscopic image of the BSPI gel; (**e**) Stereomicroscopic image of the BCs gel; (**f**) Stereomicroscopic image of the BSt gel.

Thus, the samples that showed the best homogeneity and fineness were those with added sodium caseinate and starch, followed by the sample with soy protein isolate, while the control samples, with chickpea flour and lupine concentrate showed porous structures, less fine, with numerous gaps. The gaps in the products, both in terms of their number and size, were more evident in the case of samples containing chickpea flour, lupine concentrate and in the case of the control sample.

The homogeneous, low-porosity appearance of the samples with added sodium caseinate and starch can be explained by the gelatinization of starch and the gelation of caseinate during heating. The gel solutions formed filled the gaps between the meat and fat particles constituting the meat emulsions and, together with the protein gel solutions, contribute to the binding of the mass of the composition into a unitary whole. The porous, less fine and homogeneous structure of the samples containing chickpea flour and lupine concentrate may be due to the presence of fibers, which have filiform molecules, with high hydration capacity and high molecular weight, which makes them evident after the hydration process and gelling. The elimination of air gaps or with gelatin-filled gaps, in industrial practice, is achieved by using vacuum machines to fill the composition into membranes, a condition that could not be met in the experiment.

The control sample showed the most intense color, followed by the samples with soy isolate, chickpea flour and lupine concentrate. The addition of caseinate and starch led to a significant change in the color of the finished products, motivated by the large difference in color between the meat and these functional ingredients. The use of these ingredients requires the insertion of specific food additives to improve color. Additionally, plant extracts, as a source of natural antioxidants, and essential oils can be used to prevent the oxidation of meat pigments [51,52].

4. Conclusions

Rheological measurements showed that the addition of vegetable proteins (soy, chickpeas and lupine) improved the strength of meat gels, while the addition of casein and starch reduces the strength of the gel network formed by meat and fat.

Throughout the tests, G′ registered higher values than G″ which indicated a predominantly elastic behavior for all investigated samples.

The deformation angle of the emulsions was higher for the control samples with added sodium caseinate and starch, and lower for the samples with vegetable protein additions, the decreasing evolution of the rheograms with increasing temperature indicating the improvement of the elastic properties at gelation. The compositions with the highest values of δ were characterized by pronounced fluidity in the case of emulsions and by the lowest gel rigidity.

Linear correlations were observed between denaturation temperatures of samples and cooking loss.

The cutting force decreased for all additions and recorded the lowest values for compositions with added casein and starch, the hardness being lower for all samples compared to control.

All additions decreased the cooking losses, the lowest being recorded in the samples with starch and casein, followed by the samples with added vegetable proteins. Cooking losses were correlated with cutting forces.

Investigation of the microscopic structure showed that increasing the number of fibers in the gels led to the porous and less homogeneous structures.

Due to the complexity of the derivatives used, it is appropriate to carry out further studies to establish additional correlations between the rheological behavior and yield or other indicators of interest.

Author Contributions: Conceptualization, D.I. and M.M.; methodology, D.I. and L.P.; software, L.P.; validation, M.M.; investigation, F.C., N.D. and I.V.; writing—original draft preparation, F.C.; writing—review and editing, D.I. and L.P.; supervision, M.M.; project administration, D.I.; funding acquisition, D.I. and M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania within the internal project "Obtaining an innovative preparation of minced beef, with the addition of fibers from local sources"—FiberBeef, 1066/15.06.2022.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rakosky, J. Soy products for the meat industry. *J. Agric. Food Chem.* **1970**, *18*, 1005–1009. [CrossRef]
2. Foegeding, E.A. Gelation in meat batters. *Proc. Recip. Meat Conf.* **1988**, *41*, 44–47. Available online: https://earthwormexpress.com/wp-content/uploads/2020/04/gelation-in-meat-batters.pdf (accessed on 4 July 2022).
3. Totosaus, A.; Montejano, J.G.; Salazar, J.A.; Guerrero, I. A review of physical and chemical protein-gel induction. *Int. J. Food Sci. Technol.* **2002**, *37*, 589–601. [CrossRef]
4. Hall, G.M. *Methods of Testing Protein Functionality*; Blackie Academic & Professional: London, UK, 1996. [CrossRef]

5. Bohrer, B.M. An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Sci. Hum. Wellness* **2019**, *8*, 320–329. [CrossRef]
6. Akesowan, A. Quality characteristics of light pork burgers fortified with soy protein isolate. *Food Sci. Biotechnol.* **2010**, *19*, 1143–1149. [CrossRef]
7. Serdaroğlu, M.; Özsümer, M.S. Effects of soy protein, whey powder and wheat gluten on quality characteristics of cooked beef sausages formulated with 5, 10 and 20% fat. *Electron. J. Pol. Agric. Univ.* **2003**, *6*, 3. Available online: http://www.ejpau.media.pl/volume6/issue2/food/art-03.html (accessed on 17 May 2022).
8. Jalal, H.; Mir, S.; Wani, S.A.; Sofi, A.H.; Pal, M.A.; Rather, F. Development of low fat meat products. *Int. J. Food Nutr. Saf.* **2013**, *4*, 98–107. Available online: https://www.researchgate.net/publication/271208161 (accessed on 6 August 2022).
9. Dexter, D.; Sofos, J.; Schmidt, G. Quality Characteristics of Turkey Bologna Formulated with Carrageenan, Starch, Milk and Soy Protein. *J. Muscle Foods* **1993**, *4*, 207–223. [CrossRef]
10. Urbonaite, V.; de Jongh, H.; van der Linden, E.; Pouvreau, L. Water holding of soy protein gels is set by coarseness, modulated by calcium binding, rather than gel stiffness. *Food Hydrocoll.* **2015**, *46*, 103–111. [CrossRef]
11. Raj, J.D.; Nelson, J.A.; Rao, K.S.P. A Study on the Effects of Some Reinforcers to Improve Performance of Employees in a Retail Industry. *Behav. Modif.* **2006**, *6*, 848–866. [CrossRef]
12. Gao, X.-Q.; Kang, Z.-L.; Zhang, W.-G.; Li, Y.-P.; Zhou, G.-H. Combination of κ-Carrageenan and Soy Protein Isolate Effects on Functional Properties of Chopped Low-Fat Pork Batters During Heat-Induced Gelation. *Food Bioprocess Technol.* **2015**, *8*, 1524–1531. [CrossRef]
13. Khalafalla, F.A.; Ali, F.H.M.; Abdel-Azeem, A.H.; Kassem, G.M.A.; Emara, M.M.T. Quality and acceptability of value-added beef burger. *J. Vet. Med. Res.* **2010**, *20*, 181–187. [CrossRef]
14. Das, A.K.; Anjaneyulu, A.S.R.; Verma, A.K.; Kondaiah, N. Effect of levels of full-fat soy paste on quality of goat meat patties. *J. Food Sci. Technol.* **2007**, *44*, 323–326. Available online: https://www.researchgate.net/publication/224968566_Effect_of_levels_of_full-fat_soy_paste_on_quality_of_goat_meat_patties (accessed on 7 July 2022).
15. Nasrollahzadeh, M. Biopolymer-based metal nanoparticle chemistry for sustainable applications. *Elsevier Sci.* **2021**, *1*, 56–58.
16. Gençcelep, H.; Anil, M.; Saricaoğlu, F.T.; Ağar, B. The Effects of Different Modified Starches on Some Physical and Texture Properties of Meat Emulsion. *GIDA/J. Food* **2017**, *42*, 773–786. [CrossRef]
17. Jiménez-Colmenero, F.; Herrero, A.; Pintado, T.; Solas, M.; Ruiz-Capillas, C. Influence of emulsified olive oil stabilizing system used for pork backfat replacement in frankfurters. *Food Res. Int.* **2010**, *43*, 2068–2076. [CrossRef]
18. Muguruma, M.; Tsuruoka, K.; Katayama, K.; Erwanto, Y.; Kawahara, S.; Yamauchi, K.; Sathe, S.; Soeda, T. Soybean and milk proteins modified by transglutaminase improves chicken sausage texture even at reduced levels of phosphate. *Meat Sci.* **2002**, *63*, 191–197. [CrossRef]
19. Salcedo-Sandoval, L.; Cofrades, S.; Ruiz-Capillas, C.; Matalanis, A.; McClements, D.J.; Decker, E.A.; Jiménez-Colmenero, F. Oxidative stability of n-3 fatty acids encapsulated in filled hydrogel particles and of pork meat systems containing them. *Food Chem.* **2015**, *184*, 207–213. [CrossRef]
20. Nascimento, L.G.L.; Casanova, F.; Silva, N.F.N.; de Carvalho Teixeira, A.V.; de Carvalho, A.F. Casein-based hydrogels: A mini-review. *Food Chem.* **2020**, *314*, 126063. [CrossRef]
21. Kumar, D.; Chatli, M.K.; Singh, R.; Mehta, N.; Kumar, P. Effects of incorporation of camel milk casein hydrolysate on quality, oxidative and microbial stability of goat meat emulsion during refrigerated (4 ± 1 °C) storage. *Small Rumin. Res.* **2016**, *144*, 149–157. [CrossRef]
22. Choudhury, D.; Singh, S.; Seah, J.S.H.; Yeo, D.C.L.; Tan, L.P. Commercialization of Plant-Based Meat Alternatives. *Trends Plant Sci.* **2020**, *25*, 1055–1058. [CrossRef] [PubMed]
23. Duranti, M. Grain legume proteins and nutraceutical properties. *Fitoterapia* **2006**, *77*, 67–82. [CrossRef] [PubMed]
24. Karaca, A.C.; Low, N.; Nickerson, M. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Res. Int.* **2011**, *44*, 2742–2750. [CrossRef]
25. van de Noort, M. Lupin: An Important Protein and Nutrient. In *Sustainable Protein Sources*; Academic Press: Cambridge, MA, USA, 2017; pp. 165–183. [CrossRef]
26. Lara-Rivera, A.H.; García-Alamilla, P.; Lagunes-Gálvez, L.M.; Macias, R.R.; López, P.M.G.; Natera, J.F.Z. Functional Properties of *Lupinus angustifolius* Seed Protein Isolates. *J. Food Qual.* **2017**, *2017*, 8675814. [CrossRef]
27. Archer, B.J.; Johnson, S.K.; Devereux, H.M.; Baxter, A.L. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. *Br. J. Nutr.* **2004**, *91*, 591–599. [CrossRef]
28. Keeton, J.T. Fat substitutes introduction and fat modification in processing. *Annu. Recipr. Meat Conf. Proc.* **1991**, *44*, 79–91. Available online: https://meatscience.org/docs/default-source/publications-resources/rmc/1991/fat-substitutes-and-fat-modification-in-processing.pdf?sfvrsn=2 (accessed on 18 September 2022).
29. Kaur, R.; Prasad, K. Technological, processing and nutritional aspects of chickpea (*Cicer arietinum*)—A review. *Trends Food Sci. Technol.* **2021**, *109*, 448–463. [CrossRef]
30. Ghribi, A.M.; Ben Amira, A.; Gafsi, I.M.; Lahiani, M.; Bejar, M.; Triki, M.; Zouari, A.; Attia, H.; Besbes, S. Toward the enhancement of sensory profile of sausage "Merguez" with chickpea protein concentrate. *Meat Sci.* **2018**, *143*, 74–80. [CrossRef] [PubMed]
31. Aydemir, L.Y.; Yemenicioğlu, A. Potential of Turkish Kabuli type chickpea and green and red lentil cultivars as source of soy and animal origin functional protein alternatives. *LWT* **2013**, *50*, 686–694. [CrossRef]

32. Sanjeewa, W.T.; Wanasundara, J.; Pietrasik, Z.; Shand, P.J. Characterization of chickpea (Cicer arietinum L.) flours and application in low-fat pork bologna as a model system. *Food Res. Int.* **2010**, *43*, 617–626. [CrossRef]
33. AOAC. Ch 39 Meat and Meat Products. In *Official Methods of Analysis*, 18th ed.; Horwitz, W., Latimer, G.W., Eds.; Association of Official Analytical Chemists International: Gaithersburgs, MD, USA, 2006; pp. 1–6. ISBN 0-935584-77-3.
34. Ding, Y.; Liu, R.; Rong, J.; Liu, Y.; Zhao, S.; Xiong, S. Rheological behavior of heat-induced actomyosin gels from yellowcheek carp and grass carp. *Eur. Food Res. Technol.* **2012**, *235*, 245–251. [CrossRef]
35. Patrascu, L.; Banu, I.; Vasilean, I.; Aprodu, I. Effects of Germination and Fermentation on the Functionality of Whole Soy Flour. *Bull. UASVM Anim. Sci. Biotechnol.* **2016**, *73*, 126–134. [CrossRef] [PubMed]
36. Ziegler, G.R.; Acton, J.C. Mechanisms of gel formation by proteins of muscle tissue. *Food Technol.* **1984**, *38*, 77–81. Available online: https://agris.fao.org/agris-search/search.do?recordID=US19850047665 (accessed on 3 September 2022).
37. Westphalen, A.D.; Briggs, J.L.; Lonergan, S.M. Influence of muscle type on rheological properties of porcine myofibrillar protein during heat-induced gelation. *Meat Sci.* **2006**, *72*, 697–703. [CrossRef] [PubMed]
38. Tornberg, E. Effects of heat on meat proteins—Implications on structure and quality of meat products. *Meat Sci.* **2005**, *70*, 493–508. [CrossRef] [PubMed]
39. Hamm, R. Possibilities of water binding during cooked sausage production. *Fleischwirtschaft* **1973**, *53*, 73–79.
40. Hidayat, B.; Wea, A.; Andriati, N. Physicochemical, sensory attributes and protein profile by SDS-PAGE of beef sausage substituted with texturized vegetable protein. *Food Res.* **2017**, *2*, 20–31. [CrossRef]
41. Das, A.K.; Anjaneyulu, A.; Gadekar, Y.; Singh, R.; Pragati, H. Effect of full-fat soy paste and textured soy granules on quality and shelf-life of goat meat nuggets in frozen storage. *Meat Sci.* **2008**, *80*, 607–614. [CrossRef]
42. Liu, H.; Xiong, Y.L.; Jiang, L.; Kong, B. Fat reduction in emulsion sausage using an enzyme-modified potato starch. *J. Sci. Food Agric.* **2008**, *88*, 1632–1637. [CrossRef]
43. Paternina, A.L.; Salcedo, J.G.; Romero, P.E.; Paula, C.D. Effect of Addition of Starch and Yam Flour on the Sensory and Instrumental Properties of Low Fat Sausages. *Adv. J. Food Sci. Technol.* **2018**, *15*, 166–178. [CrossRef]
44. Pătraşcu, L.; Ianiţchi, D.; Dobre, I.; Alexe, P. Effect of tumbling time and injection rate on the processing characteristics, ten-derness and color of pork biceps femoris muscle. *Ann. Univ. Dunărea Jos Galaţi, Fascicle VI Food Technol.* **2011**, *35*, 9–18. Available online: https://www.researchgate.net/publication/234137180 (accessed on 27 July 2022).
45. Cucoaneş, D.I. Research on Chemical and Enzymatic Tenderization of Meat. Doctoral Dissertation, Dunărea de Jos University, Galaţi, Romania, 2009. Available online: http://10.11.10.50/xmlui/handle/123456789/2629 (accessed on 11 August 2022).
46. Ianiţchi, D.; Diaconescu, C.; Vlad, I.; Nistor, L.; Hodoşan, C.; Marin, M.; Maftei, M. The influence of auxiliary materials on hardness, heat treatment losses and sensory properties of the meat products. *Sci. Pap. Ser. D Anim. Sci.* **2019**, *LXII*, 296–301. Available online: http://animalsciencejournal.usamv.ro/pdf/2019/issue_2/Art47.pdf (accessed on 21 September 2022).
47. Şükrü, K.; Osman, K. The Effects of Cereal and Legume Flours on the Quality Characteristics of Beef Patties. *Kafkas Univ. Vet. Fak. Derg.* **2012**, *18*, 725–730. [CrossRef]
48. Genccelep, H.; Saricaoglu, F.T.; Anil, M.; Agar, B.; Turhan, S. The effect of starch modification and concentration on steady-state and dynamic rheology of meat emulsions. *Food Hydrocoll.* **2015**, *48*, 135–148. [CrossRef]
49. Bushway, A.A.; Belyea, P.R.; True, R.H.; Work, T.M.; Russell, D.O.; McGann, D.F. Potato Starch and Flour in Frankfurters: Effect on Chemical and Sensory Properties, and Total Plate Counts. *J. Food Sci.* **1982**, *47*, 402–404. [CrossRef]
50. Hachmeister, K.; Herald, T. Thermal and rheological properties and textural attributes of reduced-fat turkey batters. *Poult. Sci.* **1998**, *77*, 632–638. [CrossRef]
51. Ben Akacha, B.; Švarc-Gajić, J.; Elhadef, K.; Ben Saad, R.; Brini, F.; Mnif, W.; Smaoui, S.; Ben Hsouna, A. The Essential Oil of Tunisian Halophyte *Lobularia maritima*: A Natural Food Preservative Agent of Ground Beef Meat. *Life* **2022**, *12*, 1571. [CrossRef]
52. Ben Hsouna, A.; Michalak, M.; Ben Akacha, B.; Dhifi, W.; Ben Saad, R.; Brini, F.; Mnif, W. Assessment of the phytochemical composition, antimicrobial activity and anti-inflammatory effects of Lobularia maritima extracts on lipopolysaccharide-stimulated RAW 264.7 cells and their capacity to extend the shelf life of raw minced beef. *J. Funct. Foods* **2022**, *99*, 105327. [CrossRef]