Effect of Process Parameters on Quality of Alfalfa Block

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To address the imbalance in the supply of grass resources caused by seasonality and regional factors, it is crucial to efficiently store and transport alfalfa. Exploring suitable grass feed processing techniques contributes to the stable transportation of grass blocks and long-term storage of nutritional components. The Central Composite Design response surface design was used to design experiments, with moisture content and compressive force as the test factors. Based on the experimental results, it was found that lower moisture content and a certain compressive force were beneficial for the stability, high density, and protein storage of alfalfa blocks. The microscopic examination of alfalfa particles revealed that a certain moisture content (15%) facilitates the formation of solid bridges between particles, leading to more stable alfalfa blocks. The final optimized process parameters were moisture content of 14.3% and compressive force of 34.8 kN. Under these conditions, the density of the molded alfalfa block was 1001 kg/m^3, with R-CP at 96.96%, R-EE at 67.23%, and R-CF at 114.13%.

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INTRODUCTION

The availability of grass resources in northern China is scarce during winter and spring, but abundant during summer and autumn (Wang 2021). These seasonal imbalances in the supply and demand of grass resources have posed a major challenge for the stability of the livestock industry. Ensuring a stable supply of grass resources is crucial for the industry’s sustainability. One of the main solutions is to process high-quality forage grass into grass feed, which could effectively address the issue of seasonal imbalance in grass resources (Tumuluru *et al.* 2018; Du 2019). Alfalfa, known as the “king of forage grass,” is rich in protein, fat, starch, and other nutrients (Feng *et al.* 2022). One common method of processing alfalfa into grass feed is by compacting it into alfalfa blocks using compacting equipment. The compression process has played a crucial role in the quality, energy consumption, and stability of processing for forage. Different biomass compression techniques also vary (Whittaker and Shield 2017; Stojkov *et al.* 2023).

Currently, the main process parameters include moisture content, compression force, and particle size (Stojkov *et al.* 2023). Moisture content plays a crucial role in preserving the quality and stability of the forage blocks over time (Hansted *et al.* 2016; Ma *et al.* 2020). Excessive moisture content could create an ideal environment for microbial growth and enzyme activity (Zheng *et al.* 2011), leading to the decay and degradation of forage blocks (Tumuluru *et al.* 2011). Additionally, it reduces the adhesive strength between particles, thereby weakening the overall structural integrity of the forage blocks (Akbari *et al.* 2021). During the production of alfalfa feed blocks, it had been observed that as the moisture content increases within the range of 15% to 25%, the density of the formed blocks decreased (Du 2023).

Compression forces also play a crucial role in determining the stability of forage blocks. Proper compression force is necessary to create a dense and compact block structure, which helps to maintain the integrity and stability of the forage blocks during storage. An increasing compression force can result in an increasing pellet density, and it was shown that pressures above 250 MPa resulted only in a minor increase in pellet density; the pelletizing pressure was shown to be dependent on biomass species, temperature, moisture content, and particle size (Stelte *et al.* 2011). The higher the binder concentration and compacting pressure, the better the briquettes, and this results in higher quality briquettes for both storage and transportation (Aransiola *et al.* 2019). With the increase in compressive force, the lower will be the porosity of the molding block, and the higher will be the stability of the molding block (Siyal *et al.* 2023). However, the high density requires higher energy consumption, which is not in line with the economic benefit demand (Du *et al.* 2022).

To enhance work efficiency and reduce energy consumption, the actual process involved compacting and forming alfalfa straw into dense blocks. Detailed screening of materials is not necessary, as long as the particle size was smaller than a certain dimension (Sudhagar *et al.* 2006). Therefore, finding the optimal balance between moisture content and compression force is essential for maximizing the shelf life of alfalfa forage blocks. This requires careful monitoring and control of the moisture content during the production process (Saeed *et al.* 2021), as well as applying the appropriate compressive force to achieve a compact and stable block structure. Through ensuring the correct combination of moisture content and compressive force, the preservation rate of key nutritional components (crude protein, crude fiber, crude fat) (Jia *et al.* 2023) in alfalfa feed blocks could be significantly improved, allowing for prolonged storage and availability of high-quality forage for animals.

EXPERIMENTAL

Materials

The alfalfa straw samples were obtained from the experimental field of Inner Mongolia Agricultural University, Hohhot, Inner Mongolia Autonomous Region, China. Briefly, alfalfa straw was harvested at florescence and then placed in a factory building (temperature about 26 °C, humidity about 10%) for ventilation and shading. After waiting for the moisture content to be less than 10%, the material underwent crushing using a 550 three-blade disc crusher equipped with a 20 mm screen, manufactured by Xingzhifu Enterprise. Following two rounds of crushing, the resulting particle size distribution was as follows: particles smaller than 1 mm accounted for 31.5%, particles ranging from 1 to 5 mm constituted 21%, particles measuring between 5 and 8 mm represented an amount of 18.5%, particles within the range of 8 to 12 mm comprised approximately 15%, while particles spanning from 12 to16 mm made up around 14%. In the process of alfalfa block processing, the broken alfalfa particles after sufficient mixing can achieve uniform distribution of particle size, which aligned more accurately with the desired outcome. The alfalfa straw was composed of cellulose (31.6%), crude protein (21.4%), lignin (7.3%), crude fat (7.3%), and ash (10.3%).

Equipment

A self-developed compression densification molding machine was used for open compression testing of alfalfa straw, wherein the size of the compressive force was altered by adjusting the length to diameter ratio of the mold. The mold used in this process was a cylinder with a diameter of 30 mm. Steps were taken to control the water content of the alfalfa straw. Firstly, the moisture content of raw alfalfa was determined by subjecting it to a drying process at 60 °C for 6 h in a LiChen drying oven (China). Then, based on the calculation results, the required amount of water was determined and evenly applied to the alfalfa straw through fine mist spraying. The wetted straw was thoroughly mixed to ensure uniform moisture distribution. After allowing it to sit for 24 h, the moisture content of the alfalfa particles was measured using the MS-H precision forage moisture analyzer (Tuoke, China).

Design-expert 12 software was used to carry out the Central Composite Design response surface design of the test program with two factors and three levels, and the test program alpha = 1.41421. The test factors were moisture content (10%, 15%, or 20%) and compressive force (12 kN, 36 kN, or 60 kN), and the test indexes were retention of crude protein content (R-CP), retention of ether extract content (R-EE), retention of crude fiber content (R-CF), and the density of alfalfa dense molding block. The test program and results are shown in Table 1.

Table 1. Central Composite Design Response Surface Design of the Test Program and Results

No.	Moisture Content (%)	Compressive Force (kN)	Density (kg*m^{-3})	R-CP (%)	R-EE (%)	R-CF (%)
1	15	36	1003	96.92	66.01	114.66
2	15	2.6	490	89.25	68.12	114.56
3	15	36	1080	97.15	66.52	122.21
4	15	36	991	96.66	67.12	117.00
5	7.92893	36	1048	93.17	75.05	125.40
6	15	36	956	95.88	66.66	125.23
7	10	12	813	91.18	76.31	111.81
8	15	36	1000	96.88	66.45	121.00
9	10	36	1007	98.72	77.02	112.10
10	15	69.94	1024	97.46	70.46	116.12
11	20	60	1108	87.44	61.91	118.25
12	22.0711	36	997	86.85	65.45	127.94
13	20	12	731	84.41	70.7	121.84

Test Standards

The measurement of nutrient content was entrusted to Inner Mongolia Bozai Quality Inspection Technology Co., Ltd. and was based on the Chinese national standards GB/T 6433 (2006), GB/T6432 (2018), and GB/T 6434 (2006) to measure the contents of crude fat, crude protein, and crude fiber content, respectively.

Precision electronic balances with an accuracy of 0.1 g were used to measure the mass. Three measurements were made with universal digital display vernier calipers to obtain alfalfa molding block diameter and height measurements. For the calculation of alfalfa block volume, three measurements of a single block were made, and then the average value was taken, through the density formula mass divided by the volume of alfalfa molding block to obtain the density of the alfalfa molding block, the average value of three alfalfa molding blocks' density measurements was taken as the final test data point.

The compressed state of alfalfa particles was examined using a microscope (Kaliyan *et al.* 2010; Anukam *et al.* 2019) under different conditions, yielding additional knowledge regarding the influence of different methods on the physical properties of alfalfa blocks. This is a stereo microscope with a magnification of 30x, an objective lens of 1.0, and an LED lighting source for supplementary light.

RESULTS AND DISCUSSION

The Central Composite Design Response Surface Test Results

The data in Table 1 were first fitted by multiple regression using Design-Expert software to obtain the quadratic term equation, which is shown as Eqs. 1, 2, 3, and 4. The significant terms in the equation were obtained by the analysis of variance (ANOVA). Details are shown in Table 2. All mathematical models were highly significant and were judged to be reliable mathematical models.

$$\text{Density} = 1006 - 15.66A + 174.75B + 27.79AB + 19.01A^2 - 113.74B^2 \quad (1)$$

$$\text{R-CP} = 96.6 - 3.37A + 3.72B - 1.13AB - 3.27A^2 - 2.94B^2 \quad (2)$$

$$\text{R-EE} = 66.48 - 4.37A - 0.52B - 2.22AB + 2.27A^2 + 1.79B^2 \quad (3)$$

$$\text{R-CF} = 115.06 + 6.34A + 0.64B \quad (4)$$

Table 2. Quadratic Equation and the Significant Terms in the Equation

	Intercept	A	B	AB	A^2	B^2
Density	1006	-15.6606	**174.754**	27.79	19.01	**-113.74**
p-values		0.3480	**< 0.0001**	0.2473	0.2923	**0.0003**
R-CP	96.602	**-3.37348**	**3.72365**	-1.1275	**-3.271**	**-2.9435**
p-values		**0.0009**	**0.0005**	0.2306	**0.0015**	**0.0027**
R-EE	66.484	**-4.36581**	-0.517593	-2.2175	**2.2723**	**1.79238**
p-values		**0.0005**	0.4875	0.0619	**0.0199**	**0.0499**
R-CF	115.059	**6.3446**	0.642371			
p-values		**< 0.0001**	0.2501			

A represents moisture content; B represents compressive force; Bolded text indicates that the item is significant.

Results of the effect of moisture content and compressive force on molding density

The fitted regression equations on density were further analyzed statistically for errors, and Design-Expert software was used to calculate precision, multiple correlation coefficients, confidence, and accuracy, as shown in Table 3 and Eq. 1. The Adjusted R^2 value was 0.9355. This shows that the fitting mathematical model had good explanatory ability. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. This ratio of 18.945 indicates an adequate signal. This model can be used to navigate the design space.

Table 3. Results of Statistical Analysis of Errors in Density

Statistical Projects	Value	Statistical Projects	Value
Std. Dev.	44.04	R^2	0.9624
Mean	947.7	Adjusted R^2	0.9355
C.V. (%)	4.65	Adequate Precision	18.9455

The interaction law between compressive force and moisture content on density, as shown in Fig. 1, is that the compressive force was positively correlated with the density within the range of 12 to 36 kN. When the compressive force was higher than 36 kN, there was no significant change in the density. When the compressive force was lower than 24 kN, the density decreased slowly with the increase of water content, whereas when the compressive force was higher than 36 kN, the moisture content did not affect molding density. It was shown that when moisture content and compressive force interact on the process of molding density, compressive force plays an absolutely dominant role.

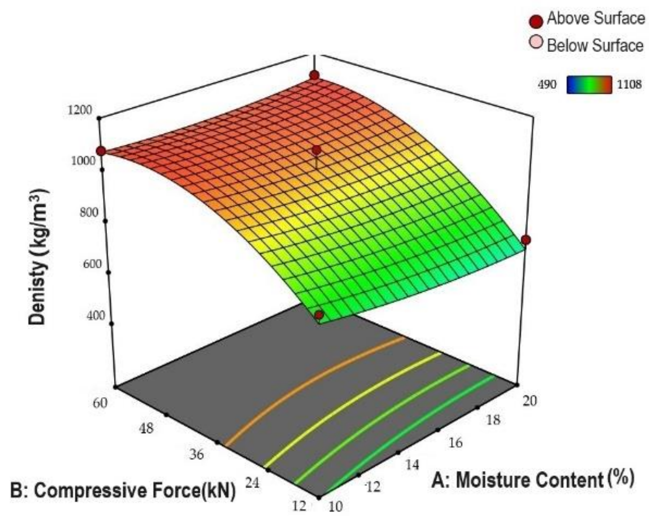

Fig. 1. The interaction law between compressive force and moisture content on density

Results of the effect of moisture content and compressive force on R-CP

As shown in Table 4 and Eq. 2, the Adjusted R^2 was 0.9112. An adequate precision value of 13.994 indicates an adequate signal. This model can be used to navigate the design space.

Table 4. Results of Statistical Analysis of Errors in R-CP

Statistical Projects	Value	Statistical Projects	Value
Std. Dev.	1.44	R^2	0.9482
Mean	93.23	Adjusted R^2	0.9112
C.V. %	1.55	Adequate Precision	13.9941

The interaction law between moisture content and compressive force on R-CP, as shown in Fig. 2, when the compressive force was in the range of 36 kN to 60 kN and the moisture content was in the range of 10% to 14%, the R-CP was higher than 95% or more. The highest R-CP could reach 98% or more, which indicated that the reasonable processing technology could make the protein content of alfalfa well stored. It would be of great benefit for the animal husbandry industry in coping with seasonal imbalance in the supply of grass feeds and in combating the shortage of grass resources caused by extreme weather. It is of great benefit for the livestock industry to cope with the seasonal imbalance of grass feed supply and combat the shortage of grass resources caused by extreme weather. Suitable moisture content is beneficial to R-CP, but it has a serious negative effect on R-CP when the moisture content is higher than a certain value; where R-CP is highest when the moisture content is between 12% and 14%, R-CP increases when the moisture content rises from 10% to that range, but in contrast, R-CP decreases when the moisture content goes from that range to 20%. With the rise of compressive force, the rise in R-CP was gradually decelerated, such that when the compressive force was higher than 36 kN, the amount of R-CP increase was almost flat.

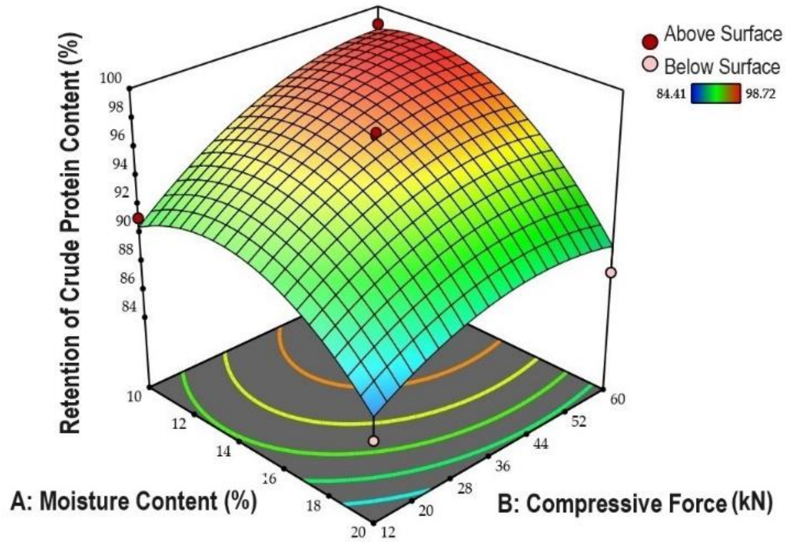

Fig. 2. The interaction law between moisture content and compressive force on R-CP

Results of the effect of moisture content and compressive force on R-EE

As shown in Table 5 and Eq. 3, the adjusted R^2 was 0.8127. This ratio of 10.2123 indicates an adequate signal. This model can be used to navigate the design space.

Table 5. Results of Statistical Analysis of Errors in R-EE

Statistical Projects	Value	Statistical Projects	Value
Std. Dev.	1.98	R^2	0.8908
Mean	69.01	Adjusted R^2	0.8127
C.V. %	2.87	Adequate Precision	10.2123

The interaction law between moisture content and compressive force on R-EE is shown in Fig. 3. The higher the compressive force, the lower the R-EE under high moisture content conditions, and the higher the R-EE with the rise of compressive force under low moisture content conditions. With the increase of water content, R-EE gradually decreased under different compressive force conditions. The results showed that water content affected the physical and chemical properties of fat, and furthermore, water content changed the effect of compression force on ether extract, thereby affecting the preservation ability of ether extract.

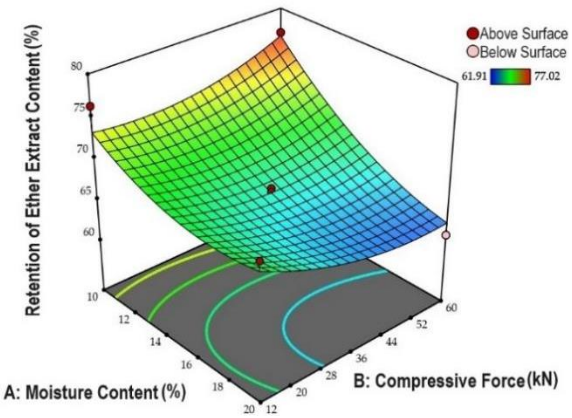

Fig. 3. The interaction law between moisture content and compressive force on R-EE

Results of the effect of moisture content and compressive force on R-CF

As shown in Table 6 and Eq. 4, the Adjusted R^2 was 0.9235. An adequate precision value of 25.1036 indicates an adequate signal. This model can be used to navigate the design space.

Table 6. Results of Statistical Analysis of Errors in R-CF

Statistical Projects	Value	Statistical Projects	Value
Std. Dev.	1.49	R^2	0.9363
an	115.06	Adjusted R^2	0.9235
C.V. %	1.29	Adequate Precision	25.1036

The interaction law between moisture content and compressive force on R-CF is shown in Fig. 4. From these results, it is obvious to find that R-CF increased linearly with the increase of moisture content; from the bottom projection contour of the 3-dimensional map, it can be seen that the compressive force had a slight effect on R-CF, *i.e.*, the compressive force gradually increased, and R-CF gently increased. This indicates that the higher the moisture content, the higher the crude fiber content in the final alfalfa block.

Optimized processing of feed stuffs

The results of the effects of moisture content and compression force on the densification of alfalfa can be judged from the density, R-CP, R-EE, and R-CF evaluation indexes mentioned above. It can be clearly found that moisture content was particularly important relative to the results of the densification process, and the value of moisture content must be considered in the forage processing process whether it is favorable to the storage and transportation of the forage. Compressive force was also found to be an important factor in feed processing. It is mainly decisive for the density of the molded feed, but also has a certain degree of influence on other indicators. Evaluation of high-density feed needs to be in the vicinity of 1000 kg/m^3, R-CP in 97 ± 0.1%, R-EE higher than 67%, and R-CF needs to be lower than 116%. Under such conditions, it is possible to obtain the optimal processing parameters as follows: moisture content of 14.3% and compressive force of 34.8 kN. Under the optimized conditions, the density of molded alfalfa cubes was

1001 kg/m^3, R-EE was 67%, and R-CF was lower than 116%. The density of molded alfalfa block was 1001 kg/m^3, R-CP was 96.96%, R-EE was 67.23%, and R-CF was 114.13%.

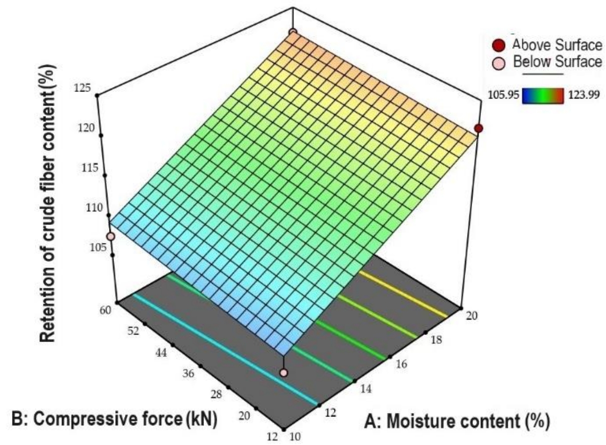

Fig. 4. The interaction law between moisture content and compressive force on R-CF

Microscopic Characteristics of Alfalfa Blocks

Observation of the state of alfalfa pellets and inter-pellets after compression can further explain the reasons for the changes in physical properties and nutrient content of alfalfa blocks under different processes. Firstly, Fig. 5(a) shows that under the water content of 8% and compressive force of 36 kN, the surface of alfalfa pellets was extruded smoothly, alfalfa stems had obvious traces of bending, and the inter-pellet portion exhibited mosaic phenomenon, but the alfalfa stems and alfalfa leaves were still clearly recognizable for their contours and morphology. Figure 5, parts (b) and (c) show the action states of alfalfa particles under the conditions of moisture of 10%, with compression force of 12 kN and 36 kN. Compared with the condition of moisture of 8%, there was no obvious difference in the appearance of interparticle action; however, under the condition of compression force of 36 kN, there was an inlay phenomenon between alfalfa stems, alfalfa stems and alfalfa leaves, and alfalfa leaves and alfalfa leaves. Figure 5 (d), (e), and (f) shows the state of alfalfa particles under the action of compressive force 2, 36, and 69 kN, respectively at 15% moisture content. In Fig. 5 (d) one can clearly observe alfalfa particles for the emergence of mosaic phenomenon. The surface of the particles was rough. This phenomenon shows that the forming pressure had a significant promoting effect on the mutual embedding process of particles. Figure 5 (e) shows that alfalfa particles within a small portion of the fiber bundles were softened and bent. The particles appeared to form a mosaic and to become entangled. In Fig 5 (f), alfalfa particles appeared similarly to Fig. 5 (e), but the alfalfa leaves were compressed and lost their original appearance. Also, multiple alfalfa leaves and part of the alfalfa stems were glued together as a whole, with no clear demarcation line between them. This suggests that the increase in compressive force promotes close contact between particles, making the alfalfa block more solid. This would further block the contact with external airborne microorganisms. Figures 5 (g) and (h) shows the state of alfalfa particles under the action of compressive force of 12 kN and 36 kN, respectively, at 20% moisture content. Figure 5 (g) shows alfalfa particles exhibiting intermediate appearance between the phenomenon of no obvious mosaic bonding. Individual particles appear to be loose, compared with Fig. 5 (b). This indicated that the high moisture content to promote the contact of the particles had a negative effect. Figure 5 (h) shows that alfalfa particles had been set together. Part of the alfalfa leaves were glued together, and it was not possible to distinguish the original contour of the particles. But for part of the fiber bundle bending, alfalfa stems almost all lost their green color and turned yellow. Figure 5(i) shows the state of alfalfa particles under the compressive force of 60 kN at 22% moisture content, and it was found that the state of alfalfa particles was similar to that of Fig. 5 (h). Only the alfalfa leaves were glued together, and the alfalfa stems were more closely adhered to the alfalfa stems.

Based on an overall comparison of Fig. 5 (a) through (i), the following advantages were found: under certain conditions of pressure, the increase of water content promoted the softening of alfalfa pellet fibers, promoted alfalfa leaf gluing, and thus was conducive to the molding of alfalfa cubes; an increase of compressive force promoted inter-pellet embeddedness, increasing the contact surface and inter-pellet bonding. The following disadvantages were found: water content above a certain level will accelerate the loss of nutrients; after the compressive force reaches 36 kN, there is no obvious difference in the state of the particles, increasing the energy consumption of compression.

Density serves as a significant indicator to assess the quality of feed pellets that have been formed. The response surface experiment's results, as depicted in Fig. 1, demonstrate a positive correlation between forming pressure and density. Moreover, an increase in moisture content leads to a slight decrease in forming density. This occurs because higher moisture content induces greater relaxation, resulting in a final stable density that is lower compared to lower moisture content. Through comparing micrographs 5(b) and 5(g), it can be observed that at the same forming pressure, the internal particles of the alfalfa pellets were more tightly packed at a moisture content of 10%, while they were looser at a moisture content of 20%.

The compressive force and moisture content had significant effects on both R-CF and R-EE. Within the range of 10% to 13% moisture content in the alfalfa, there were slight differences in R-CF. As the moisture content increased from 13% to 20%, it had a negative correlation with the impact on R-CF (Zheng *et al.* 2011). Similarly, within the range of 10% to 20% moisture content, as the moisture content increased, it had a negative correlation with the impact on R-EE. Higher moisture content was not conducive to the preservation of nutrients. When the moisture content was between 10% and 13%, the compressive force exceeded 36 kN, and the R-CF was at a high level, corresponding to alfalfa block densities higher than 1000 kg/m^3.

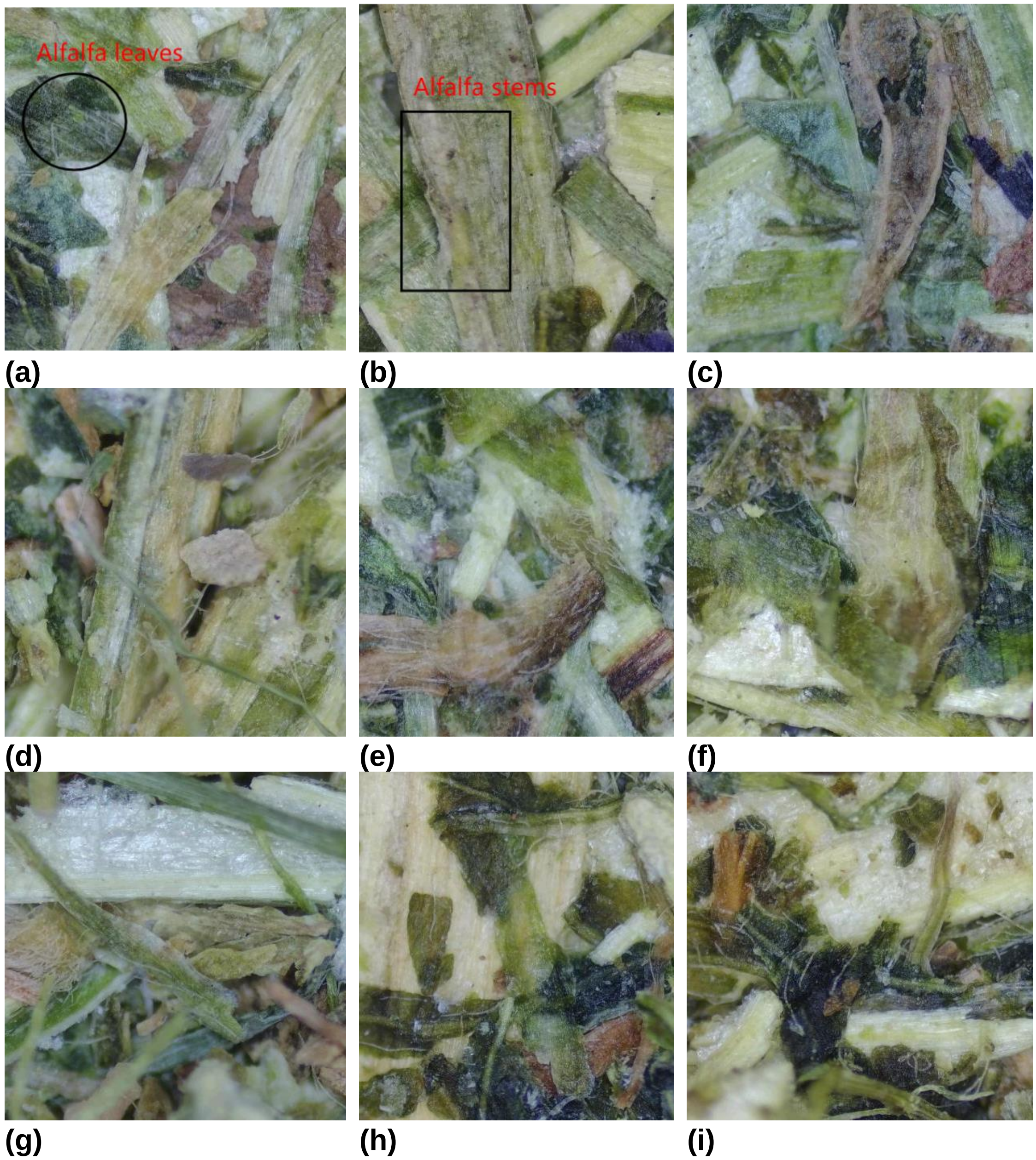

Fig. 5. Microscopic image of alfalfa after compression: (a) Compression process for 8% moisture content, compressive force 36 kN dense molding image; (b) Compression process for 10% moisture content, compressive force 12 kN dense molding image; (c) Compression process for 10% moisture content, compressive force 36 kN dense molding image; (d) Compression process for 15% moisture content, compressive force 2 kN dense molding image; (e) Compression process for 15% moisture content, compressive force 36 kN dense molding image; (f) Compression process for 15% moisture content, compressive force 69 kN dense molding image; (g) Compression process for 20% moisture content, compressive force 12 kN dense molding image; (h) Compression process for 20% moisture content, compressive force 36 kN dense molding image; (i) Compression process for 20% moisture content, compressive force 6 kN dense molding image

Figure 5 indicates that under lower moisture conditions, maintaining a compressive force that ensures a density near 1000 kg/m^3 was beneficial for the long-term storage of proteins. However, when the forming pressure was higher, it was not conducive to fat storage. Insufficient compression force can result in loosely packed blocks that are susceptible to disintegration and increased exposure to air and contaminants. The micrograph also indicates that alfalfa pellets with high moisture content exhibited severe yellowing, chlorophyll decomposition, and oxidation, further illustrating the breakdown and oxidation of fat. Materials with high forming pressure cause severe plastic deformation and cell wall destruction of alfalfa pellets, allowing the release of nutrients. High pressure forces the feed to be defatted (Shui *et al.* 2020; Guo *et al.* 2022).

Storage Performance of Loose Alfalfa

After placing the loose crushed alfalfa particles in a dry, shaded, and well-ventilated facility, the nutritional content of the particles after 6 months was as follows: crude protein content was 19.5%, crude fat content was 8.69%, and crude fiber content was 33.6%. Similarly, the values obtained for the loose material were R-CP at 91.2%, R-EE at 79.0%, and R-CF at 120.9%

A comparison of storage performance between compacted alfalfa blocks and loose alfalfa particles reveals that within a moisture content range of 10% to 15% and a forming pressure around 36 kN, compacted blocks exhibited overall superior protein storage compared to the loose storage method. However, in terms of crude fat storage, compacted blocks performed worse than the loose storage method, especially under high pressure and high moisture content conditions. The storage performance of crude fat was particularly poor in compacted blocks. The R-CF values of compacted blocks with low moisture content were lower than those of loose crushed alfalfa.

CONCLUSIONS

1. The compressive force played a decisive role in the density of alfalfa pellets, while the moisture content significantly affected the relaxation ratio of the pellets. Both compressive force and moisture content had a significant impact on R-CP and R-EE. High pressure and low moisture content were beneficial for protein storage, while low pressure and low moisture content were beneficial for fat storage. Low moisture content and high pressure were advantageous for the storage of non-fiber nutrients.
2. Using the comprehensive evaluation indicators of alfalfa block density, relaxation ratio, R-CP, R-EE, and R-CF, the authors aimed to identify the optimal densification process for a moisture content of 14.3% and a compressive force of 34.8 kN. Under the aforementioned process conditions, the density of molded alfalfa block was 1001 kg/m^3, R-CP was 97.0%, R-EE was 67.2%, and R-CF was 114.1%.
3. The results of the internal particle state of alfalfa under different processing techniques can be compared as follows: under certain pressure and moisture conditions, the alfalfa leaf particles were crushed and formed a gelatinous substance, creating adhesive bridges between particles that contributed to the stability of the alfalfa blocks. However, when the moisture content was 20%, although there was a favorable micro-

level manifestation for the formation of adhesive bridges between particles, there was also severe yellowing of the alfalfa stems, which was unfavorable for storage.

4. Under optimized processing conditions, the ability of alfalfa pellets to preserve nutritional components was higher than that of loose alfalfa particles, with the exception of crude fat.

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