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Formulation of Enhanced Animal Feed using Extrusion Processing

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In recent years, there has been a growing interest in developing innovative strategies to enhance the nutritional quality and health benefits of animal feeds. Phytogenic compounds derived from plants have demonstrated various bioactive properties, including antimicrobial, antioxidant, and immunomodulatory effects. However, incorporating these compounds into animal feeds while preserving their efficacy poses a challenge due to their sensitive nature. The current study focuses on the formulation of bioactive feed extrudates containing phytogenic compounds through extrusion processing to address this challenge. Specifically, a combination of essential oils from oregano, rosemary, chamomile, and hypericum (EOB) was examined and integrated into the standard feed through a co-rotating twin-screw extruder. The bioactive feed formulation was analyzed and compared to the conventional corn flour-based feed before and after the extrusion process. All extrudates were thoroughly evaluated regarding extrusion efficiency, morphology, physicochemical characteristics, bioactivity, and EOB release rate. Results revealed that the bioactive extrudates exhibited satisfying overall behavior concerning stability and mechanical properties. This study reveals the feasibility of creating enhanced feed by incorporating the essential oil blend (EOB) studied in the conventional feed blend through extrusion cooking. These findings contribute significantly to the ongoing efforts towards the replacement of synthetic feed additives through a novel and sustainable approach.

1. Introduction

In recent years, concerns have escalated regarding the health implications associated with animal feeds containing synthetic additives, such as antibiotics and preservatives. These additives, while aiding in feed efficiency and disease prevention, have raised significant concern due to their potential links to major health issues in both animals and consumers (Ma et al., 2021). The need for more sustainable and health-conscious alternatives in animal nutrition has thus become imperative. Addressing this concern requires a shift towards the incorporation of natural additives, which offer the potential to enhance feed quality without compromising on nutritional standards. Medicinal plants and their derivatives, particularly essential oils (EOs), represent a very promising alternative towards synthetic preservative substitution, contributing to the enhancement of animal production and health (Akram et al., 2021). Essential oils are complex mixtures of low molecular weight compounds, primarily valued for their exceptional healing properties attributed to their diverse array of biologically active components, including phenolics and terpenoids (Giannenas et al., 2013). Within this framework, the essential oils of oregano (Origanum vulgare), rosemary (Rosmarinus officinalis), hypericum (Hypericum perforatum), and chamomile (Matricaria chamomilla) were further investigated for their remarkable biological and pharmaceutical activities (Diass et al., 2021). Numerous studies have highlighted their preservative, antioxidant, antibacterial, anti-inflammatory, antiviral, and antifungal properties (Skotti et al., 2014; Ruiz-Gutiérrez et al., 2017). However, in order to include such compounds in the feed manufacturing process, a suitable technique should be employed. Several methods have been reported for the formulation of animal feed, such as pelleting, grinding, pressure cooking, extrusion, and mixing, in order to achieve optimal nutrient balance and digestibility. Among these, extrusion cooking, as a high-temperature short-time (HTST) heating process, stands out in the development of high-quality, consistent feed products (Antonio R. G. Monteiro et al., 2016; Diego R. Marques et al., 2017). More specifically, co-rotating twin-screw extrusion

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has gained scientific interest as a versatile and efficient process for feed development (Arribas et al., 2019; Kour et al., 2022). Beyond its use in the food sector, this technique has been applied as an encapsulation method for volatile, unstable, and organic compounds, such as essential oils, in the pharmaceuticals and cosmetics industries. Moreover, this method involves the simultaneous mechanical and thermal treatment of feed components, leading to improved digestibility and bioavailability of nutrients (Arribas et al., 2019). Additionally, this technology offers advantages such as precise control over the feed composition, reduction of anti-nutritional factors, and enhanced product uniformity. Even though extrusion cooking has been thoroughly studied (Yu et al., 2013), the incorporation of essential oils for the development of naturally bioactive extrudates through co-rotating extrusion is a much less explored field of research. In light of the growing awareness of the drawbacks associated with synthetic additives in animal feeds, this study focuses on the utilization of co-rotating twin-screw extrusion for the development of animal feed enriched with natural additives. The multifaceted benefits of this extrusion technique make it a promising avenue for sustainable and health-oriented animal nutrition practices. Through an exploration of natural additives and innovative extrusion cooking methods, this research seeks to contribute to the development of safer and more environmentally friendly animal feeds.

2. Materials & Methods

2.1 Materials

Oregano, Rosemary, Chamomile and Hypericum essential oils (EOs) were purchased from Herbstore S.A, Greece. Yellow corn flour (BIO-HEALTH Ltd, Greece) and soya flour (BIO-HEALTH Ltd, Greece) consisted the formulation basis of all extrudates. All reagents used for the experiments were of analytical grade.

2.2 Feed Blends Formulation

Two feed blends, one containing the selected bioactive essential oils (EOB), as well as a conventional (control) one, were developed accordingly as presented in Table 1. The moisture content of the blends was adjusted according to the experimental design, and determined using a standard method ((AOAC), 2000). Each formulated blend was mixed thoroughly, and then sealed under vacuum in PA/PE bags and stored until experimentation time. Additionally, the bioactivity of the samples was measured by quantifying their total phenolic content before the extrusion process.

Table 1: Feed blends characteristics

No	Coding	Corn Flour (%)	Soy Flour (%)	Bioactive Content (%)	Humidity (%)
1	XC	50	33.00	0.00	17.00
2	XP	50	31.80	0.20	18.00

2.3 Extrusion Processing

Extrusion was performed in a co-rotating twin-screw extruder (Prism Eurolab, model KX-16HC, Staffordshire, UK). The length, diameter, and maximum rotation speed of the apparatus were 40 cm, 16 mm, and 500 rpm respectively. The cylindrical die used, had a diameter of 3mm and length of 17.5mm. The feed blends were introduced into the extruder through a volumetric feeder. During extrusion processing, screw speed was set at 200rpm, and temperature at 180ºC by electric heaters in the five temperature zones of the device. Samples were not collected until steady-state conditions were reached (20 min). Afterwards, the extrudates were collected, allowed to air-dry, and then stored in polyethylene bags for subsequent analysis.

2.4 Extrusion Efficiency (*XE*%)

The extrusion efficiency (*XE*%) of the bioactive extrudates was measured using two different techniques. Initially, for both techniques, the entrapped EOB in the XP samples was extracted by a solvent extraction method. Briefly, approximately 0.5 g of ground XP powder was placed in a test tube with 5 mL of hexane, vortexed for 2 minutes, and then centrifuged at 3000 rpm for 3 minutes. The supernatant, rich in EOB, was collected. This procedure was repeated until the entire quantity of EOB in the sample was released. For the determination of *XE*cc%, the rich-in-EOB supernatant underwent further analysis using UV-vis spectrophotometry (UV-M51, BEL Engineering, Italy) at 274 nm, and EOB was quantified by an EOB-hexane calibration curve. *XE*cc% was represented by the amount of EOB in the final XP sample, relative to its initial amount before the extrusion process, as described in Eq. (1).

Similarly, after the extraction of EOB from the XP samples, the supernatant was introduced to a rotary evaporator (Rotavapor R-200, BUCHI, US) until hexane was completely removed. The amount of EOB collected in the evaporation flask was dissolved into 2 mL of ethanol, and the Folin–Ciocâlteu reagent was assessed for the determination of Total Phenolic Content (TPC), as reported by Laina et al. (2022). XE_{TPC}% of the XP samples was calculated as the ratio of the TPC of the XP samples before and after the

extrusion process. TPC values were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry weight. XE_{TPC}% was calculated as described below in Eq. (2).

$$XE_{cc}(\%) = \frac{\text{ml of EOB after extrusion}}{\text{ml of EOB before extrusion}} \times 100 \quad (1)$$

$$XE_{TPC}(\%) = \frac{\text{TPC after extrusion}}{\text{TPC before extrusion}} \times 100 \quad (2)$$

2.5 Evaluation of Extrudates

The extruded samples were evaluated towards their bioactivity (TPC) using the Folin–Ciocâlteu reagent (Pérez et al., 2023), as well as their physicochemical properties (morphology, chemical characteristics, glass transition temperature (T_g), thickness (H_o), expansion ratio (*ER*), hardness (*E*), colour).

Morphology

The morphology of the extrudates was examined by a scanning electron microscope (SEM) (Quanta FEG 3D, FEI, Eindhoven, The Netherlands).

Attenuated Total Reflectance, Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The quantitative and chemical characteristics of the extrudates were evaluated by ATR (ATR PRO-410 - S, JASCO International Co., Ltd.) and Infrared Spectroscopy (FT-IR) (FT / IR-4200, JASCO International Co., Ltd. Japan) analysis.

Thermal Properties

A differential scanning calorimeter (DSC) (Perkin Elmer DSC 6, CT, USA), including a Pyris operation software, was used to estimate the glass transition temperature (T_g) of the extrudates. It was calculated as the temperature midpoint of the heat capacity change (Drosou et al., 2022).

Physical Properties

More specifically, the radial diameter (thickness, H_o) of the extrudates was measured by a Vernier caliper, and the expansion ratio (*ER*) was calculated by dividing it with the diameter of the die nozzle, as in Eq.(3).

$$ER = \frac{\text{thickness of extrudate (mm)}}{\text{die nozzle (mm)}} \quad (3)$$

Furthermore, hardness was assessed by the Young Modulus (*E*) determination through compression tests conducted in a universal testing machine (Zwick model Z2.5/TN1S, Germany-Ulm). Hardness (*E*) was defined as the ratio of tensile stress to tensile strain, Eq.(4), which were recorded electronically.

$$E\ (\text{N/mm}^2) = \frac{\text{tensile stress}}{\text{tensile strain}} \quad (4)$$

The colour of the extrudates was measured using MiniScan XE (Hunter Associates Laboratory Inc, Reston, Virginia), by CIE-LAB-System colour values *L**, *a**, *b** as measures of lightness, redness-greenness, yellowness-blueness.

2.6 EOB Release Profile

The release study of EOB from the XP samples in an aqueous current (pH=7), was carried for 120 min, at a temperature of 25°C. Specifically, 2 g of grinded XP samples were added to a flask containing 20 mL of liquid medium. The mixture was magnetically stirred in a thermostatic stirred water bath. Four sets of samples were used for each release experiment. At predetermined times, 1 mL of the solution was taken to quantify the released component, as described in Section 2.4. The percentage of release of the components (*R*, %) is calculated by Eq.(5).

$$R(\%) = \frac{C_t}{C_o} \times 100 \quad (5)$$

where where C_t is the amount of EOB released at each sampling time point, *t* is the release time, and C_o is the initial concentration of EOB right after the extrusion process.

3. Results and Discussion

3.1 Extrudates Evaluation

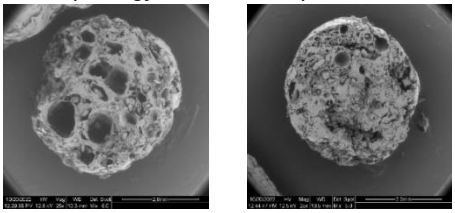
XE% of the bioactive samples, calculated using the calibration curve and TPC quantification, is presented below in Table 2. The *XE* results were adequate and in compliance with previous studies reporting values ranging from 15.5% to 86% (Amer,Rizk, 2022, Igual et al., 2022, Culețu et al., 2023). Multiple studies have emphasized the reduction in total polyphenol content observed in extrudates compared to the pre-extrusion flours, attributed to the alteration of the molecular structure of phenolic compounds at temperatures exceeding 80°C during the extrusion process (Culețu et al., 2023).

Table 2: Extrusion Efficiency (XE%) and TPC of the bioactive extrudates.

Sample	XE_{cc}%	XE_{TPC}%	TPC (mg GAE/ g dry weight)
XP	37.23 ± 0.86	36.65 ± 0.92	0.85 ± 0.09

Scanning Electron Microscopy (SEM)

The morphology of the developed XC and XP samples is presented below.



(a) (b)

Figure 1: SEM images of extrudates XC (a), XP (b)

The SEM images of the extruded samples demonstrated for both samples satisfactory results. More specifically, the XP sample appeared to be denser, with lower porosity and of slightly smaller size in comparison to the control sample. This fact is probably attributed to the presence of the essential oils, that increased the moisture content of the feed blend during extrusion, resulting in a decrease of porosity. Similar findings have been reported for corn starch extrudates by Thymi et al., 2005, where the increase in feed moisture content during extrusion resulted in a decrease in porosity values.

ATR-FTIR

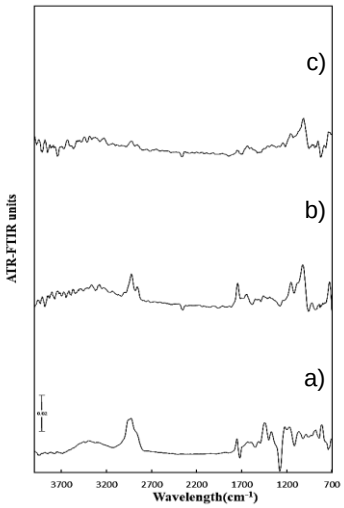


Figure 2: ATR-FTIR of EOB (a), XP (b), XC (c)

It is observed that the ATR-FTIR spectra of the XP extrudates present the characteristic peaks of both the base components (corn, soy) as well as the active ingredients (essential oils). More specifically, within the 1500-1700 cm^{-1} range, Amide I and Amide II bands reveal the presence of proteins in all samples. Carbohydrates, including starch and cellulose, exhibit characteristic peaks in the 1000-1200 cm^{-1} range. For EOB, terpenes exhibit C-H stretching vibrations in the 2800-3000 cm^{-1} range, while carbonyl groups in aldehydes and ketones may be represented by peaks within the 1600-1800 cm^{-1} range (Guzmán-Ortiz et al., 2015). Additionally, aromatic compounds contribute to the spectra with distinctive ring vibrations typically observed in the 1500-1600 cm^{-1} range. The ATR-FTIR analysis results provide valuable insight into the molecular composition of the extruded samples and demonstrate the presence of EOB in the final XP extrudates.

Physical properties

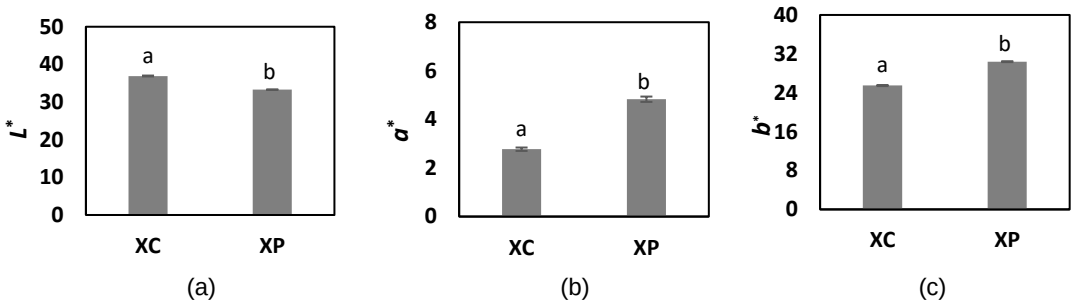
The results of the physical properties analysis of the extruded samples are presented in Table 3.

Table 3: Physical properties of the developed extrudates.

Sample	T_g	H_o (mm)	*ER*	*E* (N/mm^2)
XC	49.57 ± 1.15	4.40 ± 0.23	1.47 ± 0.11	0.36 ±0.05
XP	55.62 ± 1.63	3.30 ± 0.15	1.10 ± 0.07	0.95 ± 0.07

As demonstrated by the results presented in Table 3, XP samples exhibited lower *ER*, thickness, and *E* values compared to the control samples. The inclusion of EOB in the extrudates, owing to its liquid nature, raised the moisture content of the XP feed blend, consequently leading to reduced expansion. This finding aligns with previous studies, which have noted a significant impact of moisture content on *ER*, showing a decrease as feed moisture content increased (Thymi et al., 2005). Similarly, the addition of low molecular weight materials to a mixture with starch typically results in an overall reduction in expansion, as

suggested by Thymi et al. (2005). Moreover, highly expanded extrudates are generally achieved under conditions of high shear, pressure, and temperature within the extruder, facilitating the retention of air bubbles within the starch matrix upon expulsion from the die. This outcome is consistent with the lower E value and T_g of the XC extrudates, indicating that the more expanded samples (XC) were softer and less solid than the XP ones. Additionally, Seth et al. (2015) have also reported that higher feed moisture content correlates with increased hardness of the extrudates.



(a) (b) (c)

Figure 2: Colour parameters L (a), a* (b) and b* (c) of the developed feed samples XC, XP*

Furthermore, the colour parameter values varied significantly among the developed extrudates. Specifically, the XP samples appeared darker than the XC extrudates, exhibiting lower *L** values and higher *a** and *b** values. This difference is likely attributed to the high content of brown pigments, particularly present on the surface of the extrudates. These pigments were formed due to the extrusion parameters, notably the high temperature, which strongly affects the incorporated EOs. Since essential oils are highly sensitive to harsh environmental conditions, they are prone to partial burning, primarily at the outer layer of the extrudate that contacts the heated barrel during extrusion.

3.2 EOB Release Profile

The graphical representation of EOB's release profile from the XP extrudates is presented in Figure 3.

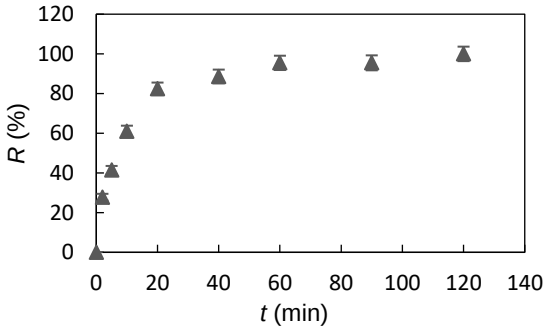


Figure 3: Release profile of EOB from the XP structures, for 120 min in aqueous medium

The release study of EOB from the bioactive extrudates, exhibited an overall gradient release rate. An initial burst effect is noted, followed by a controlled release stage. This behavior is typically linked to diffusion matrices, where progressive and partial dissolution of the matrix is observed (Palazi et al., 2018). The total release of EOB was achieved after 60min (96%). These results are in line with similar studies focusing on the sustained release of extrudates (Mansuroglu,Dressman, 2023).

4. Conclusions

The development of the innovative feed products enriched with bioactive agents presented encouraging results in terms of overall characteristics and functionality. SEM images demonstrated the morphology of the extrudates, revealing lower porosity in the XP samples. Moreover, the presence of bioactive compounds in the final feed products was confirmed through the ATR-FTIR analysis. Hardness values of the XP samples significantly increased compared to the control extrudates (XC), suggesting that the addition of EOB augmented the density of the developed XP extrudates, along with their T_g. Moreover, XP samples were characterized as darker (lower *L** and higher *b** values) than the control ones, likely due to burnt EOB particles on their surface. Finally, the bioactive extrudates demonstrated satisfactory results regarding the controlled release of EOB. Overall, this study demonstrates the feasibility of developing functional feed products with incorporated natural bioactive compounds through extrusion processing. Further research, exploring techniques to mitigate the loss of bioactive compounds and enhance the protection of bioactive ingredients during extrusion processing, will be crucial for advancing the formulation of enriched animal feed.

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