Turkish Journal of Agriculture - Food Science and Technology, 12(4): 478-483, 2024
DOI: https://doi.org/10.24925/turjaf.v12i4.478-483.6531

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Nutrient Content and *in Vitro* Digestibility of Apple Pomace Derived from Three Different Apple Cultivars

Abdulhamid Muhammad Garba[1,a], Sema Yaman Fırıncıoğlu[1,b,*]

[1]*Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences Institute, Department of Animal Production and Technologies, 51240 Niğde, Türkiye*
[2]*Niğde Ömer Halisdemir University, Agricultural Sciences and Technologies Faculty, Department of Animal Production and Technologies, 51240 Niğde, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 06.11.2023
Accepted : 15.01.2024

Keywords:
Agro-industrial waste products
Nutritional value
Apple pomace
In vitro digestibility
Ruminant feeding

ABSTRACT

This study focused on evaluating the nutritional characteristics and *in vitro* true digestibility of apple pomace derived from three apple cultivars: Golden Delicious, Starking, and Granny Smith (Malus domestica Borkh). These apple cultivars were sourced from the local market in Nigde, Turkiye. Statistical analyses, including one-way analysis of variance (ANOVA) and Duncan's test, were employed to assess variations among the apple pomace samples. Results indicated that, except for *in vitro* true digestibility, there were no significant variations in the chemical composition and total phenolic matter contents among the apple pomaces (P>0.05). However, Granny Smith apple pomace exhibited distinct features, such as higher neutral detergent fiber content (29.80%), elevated crude protein levels (5.09%) and substantial acid detergent fiber (25.30%) values. In contrast, Starking apple pomace displayed superior air-dry matter (27.24%), while Golden Delicious showcased enhanced dry matter (95.3%) and ash content (2.00%). Regarding total phenolic matter contents, Granny Smith excelled with 112.4 mg GAE/100g, outperforming Starking (103 mg GAE/100g) and Golden Delicious (75.8 mg GAE/100g). Crucially, Starking demonstrated superior *in vitro* true digestibility, with values reaching 92.36% (as received) and 92.23% (dry matter). Granny Smith, in comparison to Golden Delicious and Starking, displayed significantly different neutral detergent fiber digestibility (P<0.05). Starking apple pomace exhibit the highest overall digestibility among the apple pomaces analysed in this study, hence recommended for use in ruminant nutrition. These findings have implications for the potential utilization of apple pomace in diverse applications, given the diverse nutritional profiles of these cultivars.

[a] *abdulhamid.muhd@yahoo.com* *https://orcid.org/0000-0003-3768-3565* | [b] *semayaman60@yahoo.com* *https://orcid.org/0000-0001-9575-9981*

Introduction

Apple pomace, a byproduct of apple processing, is an abundant and underutilized agricultural waste material with the potential for various applications, including livestock feed, dietary fiber, and biofuel production (Eke-Ejiofor et al., 2018). The composition of apple pomace can vary significantly depending on the apple cultivar from which it is derived (Velderrain-Rodríguez et al., 2015). Determining the nutrient content and digestibility of apple pomace from different apple cultivars is of considerable interest, especially in the context of sustainable agriculture and waste reduction.

Apple pomace typically consists of apple peels, pulp and seeds, and its nutritional profile is characterized by the presence of dietary fiber, carbohydrates, antioxidants and essential minerals (Moure et al., 2001). However, the specific nutrient content can vary among apple cultivars, impacting its potential as a valuable resource in various industries, including animal nutrition and human health.

The *in vitro* digestibility of apple pomace is a critical aspect to assess, as it provides insight into its suitability as a feed ingredient for livestock, such as ruminants, non-ruminants, and monogastric animals (Sarnklong et al., 2010). The digestibility of apple pomace can influence its potential as a dietary fiber source and the extent to which its nutrients are available for absorption by the digestive systems of animals.

Previous studies have explored the nutritional profiles of apple pomace, shedding light on its potential applications in animal feed and as a source of dietary fiber (Kafilzadeh et al., 2008). These investigations have highlighted its rich content of dietary fiber, which can contribute to enhanced digestive health and improved nutritional balance in livestock diets. The apple variety itself plays a crucial role in determining the composition and potential utility of the pomace, making it essential to examine multiple cultivars to understand the full spectrum of possibilities.

While existing research provides valuable insights into the nutritional attributes of apple pomace, comparative analysis across different apple cultivars is limited. Understanding how apple variety affects the nutritional composition and *in vitro* digestibility of pomace can aid in optimizing its utilization in various sectors (Mertens, 2016).

This study focuses on investigating the nutrient content and *in vitro* digestibility of apple pomace obtained from three distinct apple cultivars. By examining the nutritional composition and digestibility of apple pomace derived from different apple varieties (S, GD and GS), this research aims to provide valuable insights into the utilization of apple pomace as a sustainable feed ingredient or dietary fiber source, contributing to both agricultural waste reduction and improved animal nutrition.

Materials and Methods

Collection and Preparation of Sample

The apple cultivars were purchased a local market in Nigde-Turkiye. An electric juice extractor was used for the sample extraction. Samples were milled (1mm sieve) and oven-dried at 50ºC for 48 hours before being analyzed for chemical composition and *in vitro* digestibility.

Sample Chemical Analysis

The study used established and reputable methods from AOAC (1995) to analyze the dry matter and crude ash content of apple pomaces. This choice of standardized procedures ensured the accuracy and reliability of the results, as it employed well-recognized analytical techniques for determining these components in the apple pomace samples. Crude protein in the samples was quantified using the Kjeldahl procedure, following the guidelines provided by the AOAC (1995). This involved multiplying the total nitrogen content by 6.25 to calculate CP content. The neutral detergent fiber and acid detergent fiber in apple pomaces were analyzed by Van Soest's (1991) technique. Every chemical analysis was performed in duplicate.

Total Amount of Phenolics

Using a photometric approach and the Folin-Ciocalteau reagent, the total phenolic matters content (TPC) was determined (Singleton et al. 1999). The concentration of total phenols in the apple pomace samples was measured in milligrams of gallic acid equivalents per 100 grams (mg GAE•100 g-1). This metric represents the quantity of phenolic compounds present in the samples, with gallic acid used as a reference standard. Determination of TPC involves a redox mechanism.

Determination of In Vitro Digestibilities

Samples of apple pomace from the three types of apples were subjected to digestibility testing using the DAISY^{II} incubator manufactured by ANKOM Technology (Macedon, NY), in accordance with the methodology outlined by (Goering & Van Soest, 1970). Rumen liquid from two adult Holstein cattle that were post-mortem butchered at a commercial slaughterhouse in Nigde was used to ferment AP samples, incubated at 39.5°C for 48 hours with CO_2 and buffer solutions. ANKOM^{200} fiber analyser (Ankom Technology), was used to measure NDF of the digested samples using the approach described by (Goering & Van Soest, 1970). Equations 1, 2, and 3 were used to determine the *in vitro* true DM digestibility (IVTDMD) and *in vitro* NDF digestibility (NDFD), respectively.

$$\% \text{ IVTD (as received)} = 100 - (W3-(W1\times C1)/W2\times 100 \quad (1)$$

$$\% \text{ IVTD (DM)} = 100 - (W3-(W1\times C1)/ (W2\times DM) *100 \quad (2)$$

$$\% \text{ NDFD (DM)} = 100\times[(W2\times \%NDF \text{ Feed}) - (W3 - (W1 \times C1))]/ (W2 \times \%DM \text{ Feed}) \quad (3)$$

Where W1 is the weight of the bag's tare, W2 is the weight of the sample and W3 is the bag's final weight after successive ND treatment and *in vitro* analysis, C1 = Blank bag correction (final oven-dried weight minus initial blank bag weight), Feed %NDF =% of NDF in Feed (%DM) and Feed %DM =% of dry matter in Feed.

Statistical Analysis

To ensure the reliability of the study, Statistical methods, including one-way ANOVA and Duncan's test, were employed to assess variations among the apple pomace samples. This analysis confirmed the normality, homogeneity and independence of the data. All statistical analyses were conducted using the software program SPSS.

Result

Nutritional Profile of Apple Pomace

Among the chemical parameters analyzed (with a significance level of P>0.05), there were no significant differences observed among the apple pomace samples obtained from the S, GD, and GS apple varieties (as indicated in Table 1.). However, the GS pomace sample exhibited a higher NDF value of 29.8% compared to the other two samples, which might be attributed to its thicker skin. The GD apple pomace had higher values for DM at 95.3% and ash content at 2.00% compared to the other samples. Additionally, the GS apple pomace contained more CP at 5.09% and ADF at 25.3% compared to the other apple pomaces, as shown in Table 1.

Total Amount of Phenolics Concentration

In terms of total phenolic matters concentration, there were no significant differences among the apple pomace samples (P>0.05). Nevertheless, the GS variety had the highest total phenolic matters content compared to the S and GD varieties, as shown in Table 2.

Digestibility Parameters

For *in vitro* true digestibility, there were significant differences between the apple pomaces at P<0.05. IVTD (as received) was greater in the S apple pomace at 92.36%. IVTD (on DM basis) 92.23% and NDFD 63.12% values in S apple pomace, respectively, were likewise higher. The Golden Delicious and S pomaces had considerably different (P<0.05) NDFD values than the GS (Table 3).

Table 1. Chemical content in apple pomace samples, expressed as a percentage

Apple Pomace	Air Dry matter	Dry matter	Ash	Crude Protein	Neutral Detergent Fiber	Acid Detergent Fiber
GD	25.19±0.019^{c}	95.30±0.031^{a}	2.00±0.172^{a}	2.57±0.074^{b}	28.00±0.309^{b}	22.70±1.020^{b}
S	27.24±0.133^{a}	95.10±0.028^{b}	1.47±0.082^{c}	2.02±0.021^{c}	20.70±0.291^{c}	15.90±0.131^{c}
GS	25.69±0.066^{b}	94.10±0.031^{c}	1.90±0.061^{b}	5.09±0.153^{a}	29.80±0.360^{a}	25.30±0.153^{a}

GD: Golden Delicious; S: Starking; GS: Granny Smith; There were no significant differences in the column (P>0.05) among the three apple pomace samples; Superscript (a, b, c) displays the sample that scored highest for each category

Table 2. The total phenolic matter content in apple pomaces

S/n	Apple Pomace	Total Phenolic Contents (mg GAE/100g)
1	GD	75.8
2	S	103.4
3	GS	112.4

GD = Golden delicious, S = Starking, GS = Granny Smith

Table 3. The parameters of apple pomaces in terms of their *in vitro* digestibility

Apple Pomace	*In vitro* true digestibility, %		
	As received	Dry matter	NDF
GD	88.17±0.789^{b}	87.47±0.782^{b}	57.11±4.13^{b}
S	92.36±0.386^{a}	92.23±0.311^{a}	63.12±1.81^{a}
GS	80.59±0.738^{c}	79.04±0.752^{c}	31.19±3.01^{c}

There were no significant differences in the column among the three apple pomace(P>0.05); GD = Golden delicious, S = Starking, GS = Granny Smith; NDF = Neutral detergent fiber; Superscript (a, b, c) displays the sample that scored highest for each category

Discussion

Nutritional Profile

In the analysis of the chemical composition of apple pomace samples from three distinct apple cultivars (Starking, Golden Delicious, and Granny Smith), it observed that the parameters assessed did not significantly differ from one another (P>0.05). However, it's worth noting that the DM content in these apple pomace samples, which ranged from 94% to 95%, was slightly higher compared to the 91.2% reported by Heuzé et al. (2020). Conversely, the CP content, ranging from 2% to 5%, was lower in this study compared to the 8% CP reported by Heuzé et al. (2020). Nevertheless, the CP values observed in this work, between 2% and 5%, were consistent with the 3.7% and 1.5% CP values reported by Albuquerque (2003) and Jin et al. (2002), respectively.

For ash content, the apple pomace samples in our study had levels ranging from 1% to 2%, which align with the 2.1% reported by Heuzé et al. (2020). The NDF values in our study varied from 20% to 29.8%, slightly lower than the 30% reported by Afzal et al. (2015) and substantially lower than the 36% NDF value reported by Preston (2014) for the same apple varieties (S, GS, and GD). In contrast, Heuzé et al. (2020) found a much higher NDF value of 65.1% for apple pomace. The ADF values in the samples, ranging from 15% to 25.3%, were within the range of 25.00% to 43.20% reported by Afzal et al. (2015) but lower than the 57.7% reported by Heuzé et al. (2020). Preston (2014) recorded an ADF value of approximately 27%, which is also within a similar range.

Overall, the findings indicate that the nutritional values of apple pomace samples from the three different apple cultivars fall within the ranges reported by previous researchers. It's important to consider that apple pomace's composition can vary due to factors like the ratio of skins, pulp, core, seeds, and juice, as well as apple variety, maturity, harvest season and extraction methods. These factors might account for the minor variations observed in the nutritional content of the apple pomace (Grigoraş, 2012; Kennedy et al., 1999).

This study aligns with previous research in terms of the nutritional composition of apple pomace and underscores the variability of this by-product, which can be influenced by various factors during apple processing. These findings are essential for assessing the potential applications of apple pomace in various industries, including livestock feed and dietary fiber production.

Total Phenolic Matter Content

In the TPC analysis, it was evident that the Granny Smith (GS) variety exhibited a higher TPC compared to the Starking (S) and Golden Delicious (GD) varieties. However, it's important to note that the TPC values in the study were lower than those reported in previous research. Specifically, the TPC values for apple pomace samples from GD, S, and GS were lower than the values reported by Er and Özcan (2010), which were 144, 143 and 132 mg GAE/100g and Vrhovsek et al. (2004), who reported values of 86.3, 131.1 and 121.0 mg GAE/100g. Similarly, the results of TPC from studies conducted by Bai et al. (2010) and Adil et al. (2007) indicated values of 62.7 mg GAE/100g and 47 mg GAE/100g, respectively, both of which were lower than what we observed in this study.

Overall, the total polyphenol content in the apple pomace samples examined in this study fell within the range reported by previous authors, with slight variations that could be attributed to their source. The diversity of polyphenols discovered in apple pomace is largely influenced by differences among various apple varieties and the conditions under which the polyphenols are extracted, including factors such as the type of medium, temperature, pH, and duration of extraction (Cetkovic et al., 2007). Additionally, environmental conditions can significantly impact the presence of polyphenols (Jakobek et al., 2020). Various agricultural practices, such as conventional, integrated or organic farming, can also affect the polyphenol profile (Santarelli et al., 2020). Furthermore, the location of orchards can influence the color of apples and the concentration of pigments (Yuri et al., 2019).

In essence, the findings align with the broader body of research, indicating that TPC in apple pomace can vary due to factors such as apple variety, extraction methods, environmental conditions, agricultural practices, and orchard location. Understanding these variations is essential for harnessing the potential health benefits and applications of apple pomace rich in polyphenols.

Digestibility Parameters

Only limited published studies have examined the *in vitro* true dry matter digestibility (IVTD DM) of apple pomace samples derived from various apple varieties. In this study, it observed IVTD DM values ranging from 80.59% to 92.36%. These findings closely align with previous research, such as the 84-90% range reported by Kafilzadeh et al. (2008) and Anrique et al. (2002), as well as the 82-84% range documented by Singh and Narang (1992). Notably, Tagliapietra et al. (2015) reported a 98% digestibility for fresh apple pomace after hours of incubation using the gas production method, which is in a similar range to the values observed in this study.

When examining the *in vivo* organic matter digestibility of nitrogen-treated apple pomace silage in sheep, Alibes et al. (1984) found values ranging from 70% to 78%, which closely resembles the current *in vitro* results. Furthermore, in a study involving sheep fed with dried apple pomace and supplemented only with urea, the *in vivo* dry matter digestibility was 69.9%, slightly lower than the values obtained in this *in vitro* study.

These findings collectively highlight the potential of apple pomace as a valuable feed resource, particularly in terms of its digestibility. While there are variations in digestibility between *in vitro* and *in vivo* studies, our results suggest that apple pomace can serve as a promising dietary component for livestock, providing substantial nutritional value and digestibility.

Fiber Digestibility

Only a limited number of studies have delved into the NDF digestibility (NDFD) of apple pomace. In the research, it was observed that the Granny Smith (GS) variety exhibited significantly lower NDFD, as expected, given its lower dry matter digestibility ($P<0.05$). This finding is in line with a study conducted by Ahn et al. (2002), where *in vivo* testing of apple pomace revealed an NDFD of 68.4%, a value quite close to what it was observed in the Golden Delicious (GD) variety.

Mertens (2016) emphasized the presence of a robust inverse relationship between undigested NDF (i-NDF) and *in vitro* true dry matter digestibility (IVTD-DM), suggesting that i-NDF could be a preferable analytical metric, potentially replacing NDFD, to provide a more precise understanding of variations in DM degradability among constituents. Although the current study didn't explore the correlation between i-NDF and IVTD-MD, all apple pomace samples assessed in the study exhibited higher IVTD-DM as well as NDFD.

These findings shed light on the digestibility of NDF in apple pomace, with implications for its utilization as a feed resource. The identification of GS as having lower NDFD is an important observation, offering insights into the nutritional composition of different apple cultivars.

Conclusion

The GS is lower in digestibility but higher in phenolic matters and CP content. ST has the highest IVTD and NDFD digestibility. The utilization of apple pomace in ruminant nutrition offers a promising avenue for addressing various challenges in the agricultural and animal husbandry sectors. This study has provided valuable insights into the nutritional composition and digestibility of apple pomace derived from different apple cultivars. With its potential to enhance animal nutrition, reduce feed production costs and mitigate environmental concerns associated with organic waste disposal, apple pomace emerges as a viable and cost-effective feed alternative for ruminant animals.

The wide array of apple varieties and their diverse applications further underline the versatility of apple pomace as an agro-industrial by-product. Its higher phenolic matters and protein content, coupled with the varying levels of digestibility across cultivars, opens avenues for tailored feed formulations that could optimize ruminant health and performance. While certain challenges such as variations in nutritional content and the need for further research remain, the positive outcomes from this study suggest that the integration of apple pomace into ruminant diets holds great promise.

As apple processing industries continue to generate significant amounts of pomace, tapping into this resource can not only provide sustainable feed options but also contribute to the overall efficiency of the agricultural ecosystem. However, additional research is warranted to delve deeper into aspects such as optimal inclusion levels, potential interactions with other feed ingredients and the economic viability of large-scale utilization. By embracing this innovative approach to feed supplementation, ruminant nutrition can be enhanced in an environmentally responsible and economically sound manner, ultimately benefiting both the industry and the ecosystem. In summary, the use of apple pomace in ruminant feeding can offer economic advantages by reducing feed costs and providing an alternative, value-added feed source. From an environmental perspective, it contributes to waste reduction, sustainable agriculture, and potentially renewable energy production, making it a promising option for enhancing the overall sustainability of livestock operations and the food industry. In contrast to other apple pomace varieties analyzed, Starking apple pomace demonstrates the greatest overall digestibility. This is likely attributed to specific compositional characteristics, particularly its higher concentration of soluble fiber, which has the potential to enhance digestion, making it a more suitable choice for inclusion in ruminant diets.

Acknowledgement

This manuscript derived from Muhammad Abdulhamid Garba's master thesis "Evaluation of nutritional composition and *in vitro* digestibility of apple pomace obtained from apple cultivars (Starking, Golden Delicious and Granny Smith) grown in Nigde" work at Nigde Omer Halisdemir University.

Abbreviations used

TPC, total phenolic composition; S, Starking; GD, Golden delicious; GS, Granny Smith; DM, dry matter; ADM, air dry matter; NDF, neutral detergent fiber; CP, crude protein; ADF, acid detergent fiber; IVTD, *in vitro* true digestibility; NDFD, neutral detergent fiber digestibility.

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