Assessing the physicochemical and microbiological properties of Bakery Meal used as feed ingredient in pig production

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Food losses, along the whole food supply chain, are a significant problem in Europe that affects the environment, the economy and society. According to Eurostat, nearly 59 million tonnes of food waste (131 kg per person) are generated annually in the EU, with an associated market value estimated at 130 billion [1]. Reducing food losses and reusing expired foods and food by-products is essential to achieve the EU's circular economy and sustainability goals. The circular economy is a key component of the European Green Deal, the EU's ambitious agenda for sustainable growth and climate neutrality by 2050, aiming to reduce the environmental impacts of production and consumption and to minimize waste production [2]. Along these lines, the use of expired foods and food by-products that are edible but no longer suitable for human consumption as animal feed is a viable practice, which requires strict hygiene and safety standards and faces legal and logistical challenges [3].

Bakery Meal (BM) is a type of animal feed ingredient that is made from various bakery products, which are no longer suitable for human consumption. These products include breads, crackers, chips, cookies, cereals, pastry etc. BM is produced by separating the edible parts from the non-edible ones (i.e. packaging), and then mixing, grinding, and thermally treating them. BM is mainly used as a source of energy and protein for poultry and pigs, and it can also provide some vitamins and minerals to the animals [4]. Moreover, BM is a cost-effective and environmentally friendly way of utilizing bakery waste; however, there are not many data concerning the nutritional value, the physicochemical and microbiological characteristics of bakery meal produced in Greece. Furthermore, given that the composition of the food by-products that are used for the production of each batch of BM greatly differs, BM characteristics are usually characterized by high variability [5]. The aim of this study is to assess the physicochemical and microbiological properties of different production batches of BM for its use as feed ingredient in pig production.

BM was produced at different batched, based on the availability of the bakery by-products. The by-products that comprised the raw material for the BM production were breads, cookies, pasta, cereals, chocolates, pastry, croissants etc., which were still edible but not-intended for human consumption. The portion of each by-product in the production batch varied depending on the availability of the by-products; chocolates did not exceed 10% w/w in all production batches. The by-products were collected and gathered at the BM processing plant in northern Greece, unpacked, grinded and thermally treated. The thermal treatment comprised at least 20 min thermal treatment at temperature = 133 ^{o}C and pressure = 3 bar. During the thermal treatment, the BM weight was reduced by approx. 60% w/w due to the evaporation of water. The BM, which had the appearance of a brown crumble/coarse powder, was put into 20kg bags/sacks, and stored in a dry and cool place at ambient conditions. Samples from each batch of the BM production were collected for physicochemical and microbiological analysis. The physicochemical analysis for the basic nutrients were performed according to the European Commission (EC) Regulation No 152/2009 for the following parameters: dry matter, crude protein, ash, crude fats, and crude fiber; the carbohydrates content and the gross energy were calculated based on the proximate analysis. The sugars content was measured with an enzymatic method used for the analysis of sucrose, D-fructose and D-glucose in plant and food products employing Megazyme K-SUFRG 04/18 assay kit. The concentrations of mono-, poly-unsaturated, and saturated fatty acid were also determined according to the EC Regulation No 152/20091by GC-FID (GC-2010 Plus, Shimadzu Co., Japan) employing Supelco SP2560 column, after alkaline transesterification. The Peroxide Value (PV) of lipids was determined according to the EU 2568/91 method, based on the titration of the free iodine from the oxidation of a potassium iodide solution. The amino acid profile was determined according to the EC Regulation No 152/20091. The concentration of Aflatoxins B1, B2, G1 and G2, and mycotoxins Deoxynivalenol (DON) and Zearalenone (ZON) were analysed by LC-MS/MS. Finally, the BM was also microbiologically characterized for the following parameters: Enterobacteriaceae, according to ISO 21528-2:2017, *Salmonella spp* according to ISO 6579-1:2017, *Campylobacter spp*. and *African swine fever virus* (ASFV) employing real-time PCR methods. The results are summarised in the following Table 1.

Table 1. Nutrients analysis, amino acids composition profile, aflatoxins and mycotoxins concentration, and microbiological characterization of the BM production batches. Data are presented as mean values ± SD; the data comprise five production batches and ten samples in total.

Parameter	Bakery Meal		
Nutritional analysis		**Amino acids composition (g/kg)**	
Moisture & Volatiles (g/100g)	9.25 ± 3.36	Alanine	10.86 ± 7.36
Ash (g/100g)	6.39 ± 2.91	Arginine	8.36 ± 5.33
Fat (g/100g)	19.28 ± 5.47	Aspartic acid	17.93 ± 6.45
Proteins (g/100g)	23.45 ± 6.23	Glutaminic acid	31.49 ± 16.36
Crude fibers % (g/100g)	1.07 ± 1.19	Glycine	22.73 ± 11.65
Carbohydrates (g/100g)	40.46 ± 13.81	Histidine	7.59 ± 6.45
Sugars (g/100g)	5.81 ± 5.43	Isoleucine	9.20 ± 3.59
Starch (g/100g)	23.83 ± 8.94	Leucine	13.11 ± 7.30
Energy (kcal/100g)	429.2 ± 32.6	Lysine	12.37 ± 8.86
Fatty Acid (FA) composition		Methiononine	8.43 ± 9.36
Monounsaturated FA - MUFA (%w/w)	36.2 ± 10.1	Phenyalanine	11.79 ± 3.74
Polyunsaturated FA – PUFA (% w/w)	14.4 ± 4.3	Proline	21.44 ± 21.30
Saturated FA – SFA (% w/w)	49.5 ± 17.4	Serine	11.29 ± 5.08
Iodine Value (meq O_2/kg)	< 0.5* – 9.5	Threonine	8.77 ± 4.61
Aflatoxins and Mycotoxins (µg/kg)		Tryptophane	1.05 ± 0.70
Aflatoxin B1	< 0.5* – 4.5	Tyrosine	7.00 ± 3.97
Aflatoxin B2	< 0.5* – 1.0	Valine	11.18 ± 4.67
Aflatoxin G1	< 0.5* – 1.5	**Microbiological characterization**	
Aflatoxin G2	< 0.5*	Enterobacteriaceae (cfu/g)	< 9.0* – 270
SUM of aflatoxins	< 2.0* – 7.0	*Campylobacter spp.*	ND
ZON	< 2.0* – 31	*Salmonella spp.*	ND
DON	< 40* – 244	*ASFV*	ND

* This value is the detection limit of the assay. ND: not detected.

The results show that the BM is characterised by high variability. BM can be characterised as a feed ingredient with moderate concentration of proteins (23.45 ± 6.23 g/100g), moderate to high concentration of fat (19.28 ± 5.47 g/100g), and rather high carbohydrate content (40.46 ± 13.81 g/100g), approx. half of which is due to the starch content. The nutrient characteristics of the BM resembles that of bakery products and can be used for the substitution of energy (due to carbohydrate content) and of protein sources. Concerning the amino acids profile, BM seems to comprise the essential amino acids for pig diet. More specifically, concerning the limiting amino acids, the BM contains moderate to high concentration of lysine and threonine, whereas the concentration of tryptophan is considered rather low. Concerning the concentration of aflatoxins and mycotoxins, which characterize the quality of the feed ingredients and the degree of their spoilage, they are either below the detection limit or quite lower than the limits of the EU regulation. Finally, concerning the microbiological parameters, in all production batches, no pathogens were detected (e.g. *Campylobacter spp.*, *Salmonella spp*. or *ASFV*), while the Enterobacteriaceae are either no present or in some production batches at low concentration.

Overall, the aforementioned results clearly show that BM is a valuable feed ingredient that can provide energy, protein, and other nutrients to various types of animals, including pig production. At the same time, non-edible foods are valorized as animal feed ingredients in the context of circular economy. BM composition may vary depending on the source and processing methods, so it is important to closely monitor its nutritional and safety characteristics (e.g. toxins concentration, microbiological parameters), before its inclusion as feed ingredient in pig feed ratios. Further research is needed to assess the effect of BM inclusion in pig feed on the productivity and the quality of the pig meat, as well as on the overall environmental and economical sustainability of pig production.

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