## **Quality of Old Bread as a Component for Animal Feed After Thermal Finishing in the Storage Process**

Kvaliteta staroga kruha kao komponente hrane za životinje nakon termičkih dorada u procesu skladištenja

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# QUALITY OF OLD BREAD AS A COMPONENT FOR ANIMAL FEED AFTER THERMAL FINISHING IN THE STORAGE PROCESS

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#### **SUMMARY**

After returning from the market, bread is declared as old bread and as such was once sold at a certain price  $(0.013 \in)$  and used as animal feed. Today, however, this is not allowed, but it is necessary to heat-treat this type of bread in some way. Namely, the Food Act (NN 18/2023) was passed in Croatia, which does not allow unhealthy animal feed, which includes unused old bread that is returned from the market. Based on the above, the aim of this paper is to thermally process white, corn and black bread by drying, pelleting, extruding, and getting a new component in animal nutrition. After that, store and determine whether there is an impact of heat treatment on the health of such bread. By monitoring the storage time of old bread that the sample temperature was significantly influenced by the duration of storage. During the storage process, the moisture content in the samples of old bread varied significantly. The protein content differed significantly between the types of old bread, as well as the method of processing. Microbiological analysis of processed samples shows that all samples are healthy and can be used as feed.

Keywords: types of bread, drying, pelleting, extrusion, storage, microbiological analysis

#### **INTRODUCTION**

Until about ten years ago, old bread was used as an ingredient in animal feed in the Republic of Croatia. However, with the accession to the EU, the country was forced to comply with various regulations and guidelines for the use of only healthy animal feed (Veissier et al., 2008), which includes the use of stale, unused bread returned by shops to producers (NN 115/18 NN; 18/2023).

Bread is generally the most common form of grain food. It is defined as a bakery product weighing more than 250 g, made by mixing, shaping, fermenting and baking dough (OG 81/2016). Traditionally, it is made from flour, salt, water and yeast.

However, due to the increasingly demanding market, about 20% more bakery products, including bread, are produced per capita than are consumed, resulting in a surplus of over 90,000 tons per year in Croatia (Statistical Yearbook, 2019). This bread becomes a waste that needs to be disposed of.

Since such bread needs to be disposed of, bread producers are forced to pay for the disposal of old, unused bread or heat it.

The most common heat treatment is drying, pelleting or extrusion (Putier, 1993). Drying is the process of interaction between wet material and the environment, where the result of drying is the transfer of moisture from the material to the environment (Doymaz and Pala, 2003). In this case, the amount of moisture in the material is equal to the moisture in the air and the material is considered dried (Abasi et al., 2016). Only the drying of

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the material is done with a dryer, and the type of equipment depends on the material to be dried (Voća et al., 2021). Pelletizing is a thermoplastic process of forming a material by extrusion using rollers over a matrix, in which finely divided particles of flour material are formed into compact pellets (Voća, 2008). There are different types of pelletizers, usually distinguished by how the die is placed (horizontally or vertically), how it rotates, how many rollers there are in the pelletizer, and whether there is built-in air conditioning (Kocsis and Csanády, 2019). Extrusion involves forming a softened, plasticized material by extruding it with the help of a screw, subjecting the material to high pressure, high temperature, and mechanical stress. The extrusion of the material is done with an extruder and depends on the desired result. There are dry and wet extruders (Pérez et al., 2008).

Just before heat treatment, the bread must be ground. Grinding is a mechanical process whose main objective is to reduce the size of the particles in order to increase digestibility and achieve a suitable size that allows better preparation of the materials for further processing or machining (Lyu et al., 2020). The quality of particle size is determined by the uniformity modulus, and particle sizes are divided into coarse, medium and fine. The degree of comminution that tends to be achieved is as many medium particles as possible (McElhiney, 1994). In this case, the mean particle size must be at least 68% to determine that the mill is working well (McElhiney, 1994). Indeed, the characterization of the particles has a direct influence on the flowability as well as on the shaping (pelletization, extrusion), as documented by Šklubalová and Zatloukal (2013). The grinding itself is carried out using various mills, the most common being a hammer mill or a mill with rollers (Khamila et al., 2019).

Storage is the last operation in the whole process of manufacturing a component or product. Given the purpose of storage, it is necessary to know what is stored, how, for how long (short or long term) and where. In animal feed factories, components or finished feeds are stored in warehouses or silos made of concrete or steel (Savoie and Jofriet, 2003). Factors that affect the possibility of proper storage during the required period of use are humidity and temperature of the components (products) and ambient air, and respiration of the components (Sharma, 2007). In animal feed factories, food safety is ensured by evaluating the possible effects of potential hazards that can contaminate food with undesirable substances (Sokolović and Ruk, 2011).

If the component is not well processed during storage, bacteria can grow. Therefore, microbiological analyzes are performed, based on which the nutritional and microbiological quality of the components that can affect the growth and development and health of the animals are evaluated. The most commonly studied bacteria are *Salmonella, Clostridia,* saprophytic bacteria, molds and yeasts (Samli et al., 2008). Thermally processed and stored bread proved to be a quality component in chicken diets in the studies of Krička et al. (2019) and Krička et al. (2020).

In order to use discarded waste bread as animal feed, the aim of this work is to investigate the possibility

of quarterly storage of white, corn and brown bread after thermal processing by drying, pelleting and extrusion.

#### **MATERIALS AND METHODS**

Studies were conducted on white, corn, and brown bread. The waste bread was collected as return bread from retail shops and brought to the Faculty of Agriculture in Zagreb on the same day. The waste bread was then dried in an oven (Memmert UF 55 plus, Germany) for 2 days at a temperature of 50°C and ground using a laboratory mill (CyclotecTM 1093 Sample Mill, Foss, Denmark) according to method HRN 6498: 1998. After grinding with a sieve shaker (Retsch AS 200), the uniformity modulus was determined according to the recommended procedure of ASAE (2008).

Pelletizing was carried out on a pelletizer type 14-175 (Amandus Kahl, Germany) at a granulation temperature of 100°C and a matrix size of 4 mm. Extrusion was carried out on a continuous twin screw extruder type MPF-50: 15 Baker Perkins, at an extrusion temperature of 120 °C.

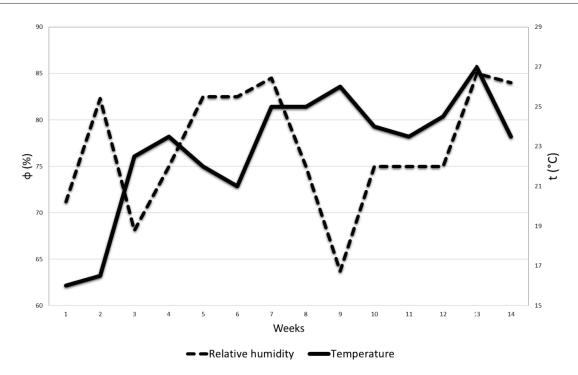
After drying, pelleting, and extruding, all three types of stale bread were packed in double-layer paper bags with a thickness of 0.16 mm, sealed with quilting thread, and then stored in a concrete floor storage on wooden pallets. The dimensions of the floor storage were 40x20x10 meters. During storage, the temperature and relative humidity in the storage room were measured once a week at 10:00 am using a psychrometer. In addition, the temperature of the stored stale bread was monitored with an i50 infrared camera (Flir Systems AB, Stockholm, Sweden). The mass of samples of heattreated stale bread was measured using a digital balance with two decimal places (Mettler Toledo PB1502), the moisture content of the sample was measured according to HRN ISO 6540: 2002 using the Memmert UF 55 plus laboratory dryer and the protein content was measured according to HRN EN ISO 5983-2: 2010 by Kjeldahl.

In addition, the health status of dried, pelleted and extruded stale bread was monitored at the beginning and during storage (4 dates in total). The presence of *Salmonella* in 50 g (HRN ISO 6579/2003), the presence of sulfidoreducing *Clostridia* in 1 g (HRN EN 7937/2005), the presence of saprophytic bacteria in 1 g (HRN ISO 4833/2008) and the presence of mold and yeast in 1 g were determined (HRN ISO 7954/2002).

The data were analyzed using the procedure of the SAS system package, version 9.4. The obtained results were processed by analysis of variance, while the differences between the means were compared by LSD test for p = 5%.

#### **RESULTS AND DISCUSSION**

In the storage process, once a week, for 14 weeks from June to August, the temperature and relative humidity in the storage area were monitored. Diagram 1 gives a graphical representation of the movement of temperature and relative humidity during the storage process of the analyzed old bread.



**Figure 1. Graphical representation of temperature and relative humidity during storage of the old bread studied** *Grafikon 1. Grafički prikaz temperature i relativne vlage zraka tijekom skladištenja proučavanoga starog kruha* 

During the 14-week summer storage period, all types of old bread (white, corn, and brown) and all types of processing (drying, pelleting, extruding) were monitored for temperature and relative humidity in the storage. The air temperature in the storage ranged from  $16^{\circ}$ C to  $27^{\circ}$ C, while the relative humidity ranged from 64% to 85%. Thus, the ability to absorb water into the air depends on the current air temperature; the warmer the air, the more water it can take on. Both variables varied as a function of each other, as noted by González-Torralba et al. (2013) in his study of wheat bread storage. The

Tablica 1. Omjer veličine čestica triju vrsta staroga kruha

variation and trend of the increase in relative humidity were determined so that the surrounding factors influenced some parameters that were monitored in the experiment (mass and humidity of the sample of the components studied).

To determine the quality of ground stale bread, the ratio of coarse, medium, and fine particles after grinding was determined, i.e. the uniformity modulus. Table 1 shows the results of screened waste bread (white, corn and brown bread).

Type of bread / Vrsta kruha	Mu	dgw	Sgw	W (%)
White / <i>Bijel</i> i	12 : 71 : 17	1.69290	1.5762	10.23
Corn / Kukuruzni	15 : 78 : 7	0.50896	1.77146	11.14
Brown / Crni	9 : 68 : 23	0.43014	1.8396	10.18

Mu – uniformity model, dgw – geometric mean diameter, Sgw – geometric standard deviation, w – moisture content / Mu – model uniformnosti, dgw – geometrijski srednji promjer, Sgw – geometrijska standardna devijacija, w – sadržaj vlage

Mills that grind components in compound feed mills must meet a number of factors during their operation. One of the most important of these is that they must grind in such a way that the particle size ratio is such that the proportion of medium particles is as high as possible and that of fine and coarse particles is as small as possible (Chewning et al., 2012; Iskenderov et al., 2018). The expected ratio is a minimum of 68% mean particles (McElhiney, 1994), therefore, in parallel with the milling of all three types of bread (white bread, corn bread, brown bread), it can be concluded that a quality bread milling was carried out. Indeed, the average particle size ranged from 68 to 78%. However, brown bread has an extremely high percentage of fine particles (up to 23%), so there is a possibility that some components may behave like dust during internal transport and cause losses. For this reason, corn bread had the best particle ratio with the lowest percentage of fine particles but also the highest percentage of medium particles. This is confirmed by Pliestić (1999) in his study on losses of feed mixtures during transport as a function of particle size. Moreover, particles of different size and size distribution also show different behaviors. For example, Jalšenjak et al. (1998) found that fine particles have higher specific surface area and bulk density and that porosity and flow rate depend significantly on particle size.

After the determination of particle sizes, the influence of 14-week storage of bread types (white bread, corn bread, brown bread) and (drying, pelleting and extrusion) on the temperature of the studied samples was determined. In addition, the mass of the old bread samples was measured during the storage period—that is, the influence of storage period and bread type, as well as of technological processing, on the mass of the samples. Table 2 shows the listed values.

Table 2. Influence of storage time, type of old bread and technological processing on the temperature and weight of samples of thermally processed old bread

Tablica 2. Utjecaj vremena skladištenja, vrste staroga kruha i tehnologije dorade na temperaturu i težinu uzoraka termički dorađenog starog kruha

Weeks of storage /	Sample temperature / p<0.001	Sample mass / p>1.000 <i>Masa uzorka</i>				
Tjedan skladištenja	Temperatura uzorka					
1	18.91 k ± 0.50	$4.57 \pm 4.05$				
2	17.76 l ± 0.51	4.58 ± 4.06				
3	21.69 i ± 0.48	4.59 ± 4.07				
4	23.43 f ± 0.45	4.59 ± 4.05				
5	21.98 h ± 0.43	4.61 ± 4.08				
6	21.13 j ± 0.26	4.61 ± 4.08				
7	24.19 d ± 0.25	4.62 ± 4.09				
8	24.93 c ± 0.18	4.62 ± 4.08				
9	25.98 a ± 0.29	4.59 ± 4.05				
10	23.93 e ± 0.82	$4.58 \pm 4.06$				
11	23.35 f ± 0.65	$4.58 \pm 4.06$				
12	$24.06 \text{ ed} \pm 0.38$	4.61 ± 4.08				
13	25.67 b ± 0.44	4.61 ± 4.08				
14	22.91 g ± 0.37	4.61 ± 4.07				
Bread type /	Sample temperature / p>0.9531	Sample mass / p>0.9624				
Vrsta kruha	Temperatura uzorka	Masa uzorka				
White / <i>Bijeli</i>	22.90 ± 2.33	4.67 ± 3.82				
Corn / Kukuruzni	22.81 ± 2.39	4.59 ± 4.18				
Brown / Crni	22.85 ± 2.32	4.53 ± 4.02				
Technological processing /	Sample temperature / p>0.4720	Sample mass / p>0.9412				
Tehnološka dorada	Temperatura uzorka	Masa uzorka				
Drying / <i>Sušenje</i>	22.72 ± 2.34	$4.62~b~\pm~0.34$				
Pelleting / Peletiranje	23.06 ± 2.44	$4.56 \text{ a} \pm 0.22$				
Extrusion / Ekstrudiranje	22.77 ± 2.23	4.59 c ± 0.11				

a-I Mean values within a column having different superscripts are significantly different by least significant difference test (p<0.05) / a-I Srednje vrijednosti unutar stupca koji ima različite superskripte značajno se razlikuju prema testu najmanje značajne razlike (p<0.05)

Observing the influence of storage time, type of old bread (white bread, corn bread, brown bread), and technological processing (drying, pelleting, extruding) on the sample temperature, it can be concluded that it was significantly (p < 0.001) influenced by the storage time, while the type of bread and technological processing had no significant influence. A similar conclusion was reached by Liu et al. (2010) in the storage of biological material during the storage of tomato powder.

Significant variations in sample temperature during the storage process can be explained by variations in environmental conditions during storage (temperature and relative humidity), which were not controlled. Similar conclusions were drawn by González-Torralba et al. (2013). In addition, storage time, bread types and technological processing did not significantly affect the mass of the samples. Although there is no significant influence, a small increase in sample weight is observed during the storage process, which can be explained by the increase in moisture in the samples due to the influence of ambient air during storage (Wrigley et al., 1994).

In addition to the above parameters, the change in moisture content in the old breads studied was monitored, as well as the change in protein content during the storage period. Table 3 shows the values of the influence of storage period, bread type and technological processing on moisture and protein content in the samples of thermally treated old bread.

#### Table 3. Influence of storage, type of bread and technological processing on moisture and protein content in samples of thermally processed old bread

Tablica 3. Utjecaj skladištenja, vrste kruha i i tehnologije dorade na sadržaj vlage i proteina u uzorcima termički dorađenoga starog kruha

Weeks of storage /	Moisture / p<0.001	Proteins / p>0.0051 NS
Tjedan skladištenja	Vlaga	Proteini
1	$9.70~d~\pm~0.89$	9.44 ± 2.85
2	9.89 dc $\pm$ 1.01	10.35 ± 0.79
3	$10.06 \text{ bdc} \pm 0.79$	10.44 ± 0.66
4	$10.06 \text{ bdc} \pm 0.81$	10.39 ± 0.61
5	10.40 bac $\pm$ 0.50	10.52 ± 0.66
6	10.59 ba $\pm$ 0.58	10.62 ± 0.80
7	10.73 a ± 0.54	10.52 ± 0.80
8	$10.57 \text{ ba} \pm 0.58$	10.60 ± 0.70
9	10.36 bac $\pm$ 0.56	10.50 ± 0.70
10	$10.08 \text{ bdc} \pm 0.53$	10.51 ± 0.70
11	10.15 bdc $\pm$ 0.60	10.54 ± 0.67
12	9.61 d ± 2.90	10.55 ± 0.62
13	10.88 a ± 0.42	10.53 ± 0.62
14	10.57 ba $\pm$ 0.61	10.59 ± 0.60
Bread type /	Moisture / p<0.001	Proteins / p<0.001
Vrsta kruha	Vlaga	Proteini
White / <i>Bijeli</i>	10.57 a ± 0.67	10.00 c ± 0.48
Corn / Kukuruzni	9.90 b ± 0.81	10.34 b ± 0.54
Brown / Crni	10.32 a ± 1.42	10.98 a ± 1.47
Technological processing /	Moisture / p<0.001	Proteins / p<0.001
Tehnološka dorada	Vlaga	Proteini
Drying / Sušenje	10.10 b ± 0.78	10.62 a ± 0.78
Pelleting / Peletiranje	9.92 b ± 0.78	10.01 b ± 1.49
Extrusion / Ekstrudiranje	10.77 a ± 0.78	10.68 a ± 0.24

a-d Mean values within a column having different superscripts are significantly different by least significant difference test (p<0.05) / a-d Srednje vrijednosti unutar stupca koji ima različite superskripte značajno se razlikuju prema testu najmanje značajne razlike (p<0.05)

During the storage process, the moisture content in the samples of old bread varied significantly (p < 0.001). This can be explained by changes in environmental conditions during storage as both temperature and relative humidity in the warehouse varied significantly (Pixton and Warburton, 1968). The type of stale bread (white bread, corn bread, brown bread) had a significant effect on the moisture content, being higher for white bread (10.57%) and brown bread (10.32%), while lower moisture content was found for corn (9.90%). In terms of technological processing (drying, pelleting, extrusion), moisture content was highest in extruded circle (10.77%), followed by dried bread (10.10%) and pelleted (9.92%).

The protein content of the samples was not affected by the storage period and ranged from 9.44% to a maximum of 10.62%. However, protein content differed significantly (p < 0.001) between old bread types and processing methods (p < 0.001). This is due to the type of cereal used for bread production, because according to Grbeša (2004), the protein content in wheat is on average 11.93% and in corn 9.20%. Bojňanská et al. (2021) indicate a protein content in fresh bread of 11 to 13%. Since food processing involves a combination of friction, heat, residence and wetting times, this can lead to partial denaturation of proteins in foods (Thomas et al., 1998), which can increase their digestibility (Voragen et al., 1995), resulting in small differences in protein content after thermal processing of bread, as seen in the study. Since the protein content in the samples of thermally processed bread did not change significantly during the storage process and the analysis of protein content was carried out at the beginning of the experiment, the protein content was determined in relation to the type of bread. This phenomenon can be explained by the above data on protein content of wheat and corn.

In parallel with these tests, microbiological analyzes of the samples at the beginning of the study and once a month, as well as at the end of storage, were carried out for salmonella, sulfide-reducing clostridia, saprophytic bacteria, and molds and yeasts. Table 4 shows the values obtained.

Sample / <i>Uzorak</i>	Salmonella in 50 g / Salmonela u 50 g				Sulfido reducing clostridia in 1 g / Sulfido reducirajuća klostridija u 1 g							Molds and yeasts in 1 g / Plijesni i kvasci u 1 g				
Month / <i>Mjesec</i>	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Dried white bread / Osušeni bijeli kruh	N	N	N	N	N	N	N	N	2000	2000	0	1000	1000 <i>Penicillium</i> sp.	0	500 <i>Penicillium</i> sp.	0
Dried corn bread / Osušeni kukuruzni kruh	N	N	N	N	N	N	N	N	0	4000	0	0	2000 <i>Penicillium</i> sp., yeasts	6500 kvasci, <i>Mucor</i> sp.	0	2000 <i>Penicillium</i> sp.
Dried brown bread / Osušeni crni kruh	N	N	N	N	N	N	N	N	4000	0	3000	4000	2000 <i>Penicillium</i> sp.		2000 <i>Penicillium</i> sp.	1500 <i>Penicillium</i> sp.
Pelletized white bread / Peletirani bijeli kruh	N	N	N	N	N	N	N	N	2000	3000	0	4000	500 <i>Rhizopus</i> sp.	0	500 <i>Penicillium</i> sp.	0
Pelletized corn bread / Peletirani kukuruzni kruh	N	N	N	N	N	N	N	N	2000	2000	2000	2000	500 <i>Rhizopus</i> sp.	0	0	0
Pelletized brown bread / Peletirani crni kruh	N	N	N	N	N	N	N	N	2000	0	0	0	0	0	0	0
Extruded white bread/ <i>Ekstrudirani bijeli kruh</i>	N	N	N	N	N	N	N	N	0	1000	1000	1000	1000 yeasts	0	0	1000 <i>Penicillium</i> sp.
Extruded corn bread / Ekstrudirani kukuruzni kruh	N	N	N	N	N	N	N	N	1000	0	0	0	0	0	0	0
Extruded brown bread / Ekstrudirani crni kruh	N	N	N	N	N	N	N	N	0	2000	1000	1000	0	0	0	0

#### Table 4. Microbiological analysis of stored old bread from the beginning to the end of storage

Tablica 4. Mikrobiološka analiza skladištenoga starog kruha od početka do kraja skladištenja

N-not detected / N-nije otkriveno

Microbiological analysis of the samples (dried white bread, dried corn bread, dried brown bread, pelleted white bread, pelleted corn bread, pelleted brown bread, extruded white bread, extruded corn bread, extruded brown bread) was carried out for the presence of salmon-red saprophytic bacteria and mold and yeast. Contamination of cereals with molds-that is, with mycotoxins—explicitly depends on environmental conditions (Mašek and Šerman, 2006). Since molds appear already at a relative humidity of 70%, this would be expected to be noticeable. From all the results of the microbiological tests, it is clear that the thermally processed old bread is hygienic and that there are no significant variations in the presence of undesirable microorganisms. Although the experiment was conducted in the warmer months (June to August), when the probability of an increase in the presence of undesirable microorganisms is greater, such changes were not detected analytically in thermally treated stale bread.

All samples analyzed during storage complied with the Regulation on the safety of feed (OG 102/2016) and were considered healthy.

Indeed, high quality feed means not only feed with an appropriate nutrient composition required for a particular animal species, but also microbiologically correct feed that does not contain bacteria and songs and other undesirable substances that can affect the health and productivity of the animals (den Hartog, 2003; Kan and Meijer, 2007).

#### CONCLUSION

In studying the effects of storing white bread, corn bread, and brown bread in concrete warehouses after heat treatment by drying, pelleting, and extruding for a period of 14 weeks, it was found that storage air had a significant effect on the temperature of all samples, while the mass of the samples was not affected. In addition, the moisture content of the samples varied significantly during the storage process, as did the protein content. From a health point of view, the microbiological analysis of the samples of all bread types (white bread, corn bread, brown bread) and all thermal treatments (drying, pelleting, extruding) for *Salmonella*, sulfidereducing *Clostridia*, saprophytic bacteria and molds and yeasts were considered healthy according to the Feed Quality Regulation.

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### KVALITETA STAROGA KRUHA KAO KOMPONENTE HRANE ZA ŽIVOTINJE NAKON TERMIČKIH DORADA U PROCESU SKLADIŠTENJA

### SAŽETAK

Nakon povrata s tržišta, kruh se deklarira kao stari i kao takav se nekad prodavao po određenoj cijeni (1kn = 0,013 €) te koristio kao hrana za životinje. Danas, međutim, to nije dopušteno, već je na neki način potrebno takav tip kruha termički obraditi. Naime, u Hrvatskoj je donesen Zakon o hrani (NN 81/2013), u kojem nije dozvoljena zdravstveno neispravna hrana za životinje, a u koju spada i neiskorišteni stari kruh koji se vraća s tržišta. Temeljem navedenoga, cilj je ovoga rada bio bijeli, kukuruzni i crni kruh termički doraditi, i to sušenjem, peletiranjem i ekstrudiranjem, te dobiti novu komponentu u ishrani životinja. Nakon toga bilo ga je potrebno skladištiti i utvrditi postoji li utjecaj termičke obrade na zdravstveno stanje takvoga kruha. Praćenjem vremena skladištenja na vrste staroga kruha i tehnološke obrade na temperaturu uzorka, može se utvrditi da je temperatura uzorka bila pod značajnim utjecajem trajanja skladištenja. Tijekom procesa skladištenja, sadržaj vlage u uzorcima staroga kruha značajno je varirao. Sadržaj proteina značajno se razlikovao između vrsta staroga kruha, kao i načina obrade. Mikrobiološkom analizom dorađenih uzoraka dokazano je da su svi uzorci zdravstveno ispravni i mogu se koristiti kao hrana za životinje.

Ključne riječi: tipovi kruha, sušenje, peletiranje, ekstrudiranje, skladištenje, mikrobiološka analiza

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