# Effect of extruding on microbial indicators of feed mixtures

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## Abstract

**Introduction.** To determine the quality of feed mixtures containing flax extract from serum was investigated microbiological parameters after extruding feed mixtures of different prescription in storage.

**Materials and methods.** Studied feed mixture of wheat, corn and flax extract on serum basis of different interest amount. Linen extract obtained by extraction in an active pulsation dispersant mixture is mixed and aperture extruding a temperature – 110–120 °C, pressure – 4.2 MPa, which allows almost completely disinfect it.

We investigated the change of microbiological feed mixtures during storage. The test samples were placed in cloth bags and stored for 2 months at 0 °C (refrigerator), + 20 °C (thermostat) and relative humidity – 45%.

**Results and discussion.** Humidity grain mixtures for extrusion samples is within 16.8–17.6%, after extruding from 13.3 to 13.5%, and over the shelf life of unchanged at +20 °C and a relative humidity of 45% designs humidity changes during storage of 13.3–13.5% to 15.3–16.1%.

Changing humidity extruded feed mixture to a large extent dependent on storage temperature and not on the number entered flax extract from the serum of the mixture.

Comparing the conditions and shelf life, it can be said that the change in humidity extruded feed mixture to a large extent dependent on storage temperature and not on the number entered flax extract from the serum of the mixture.

At the beginning and end of storage extruded forage mixture, characterized by relatively low levels of microbiological contamination. Not revealed the following groups of microorganisms as Escherichia coli (BGKP) pathogens. Total mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) in all samples of mixtures is within acceptable limits (no more than 5·105 CFU/g).

Analysis of colonies of mesophilic aerobic microorganisms on agar peptidemeat found that they are characterized by large, small and medium size, white and yellow color, smooth and rough edges. The bulk of the microorganisms is coccoid bacteria whose cells are placed singly or clusters. The main morpho types bacteria isolated from grain extrudate is aerobic bacteria.

**Conclusion.** We recommend using extrusion process as an effective way to improve health quality extruded feed mixtures because it provides a nearly sterile product.

## Introduction

Combination fodder – is much more difficult complex for keeping than grain, flour and cereals. It can be explained by the large number of components, which the fodder includes, and also by physical, chemical and biological properties of each component [10].

Different components have different critical humidity. So, the critical humidity of bone meal is 8.7%, of flour from Lucerne's leaves is 14.9%, cattle cake, solvent cake and from cottonseed is 11.5 and 12.8%. The critical humidity of feed depends of the components and can be 10.0–14.5%. Therefore, when its value is higher, than an intensification of biochemical processes and active development of microflora can happen. The combination fodder's microflora majority consists of microorganisms, which are inhabiting the grain mass [2, 8–11].

Combination fodder is a favorable nutritive ground for many microorganisms, especially for mold fungi. If there is enough level of humidity and a temperature is about 10–20 °C, then mold fungi formation happens faster, mold can produce a lot of heat, so they are the main reason of fire-fanging effect of grain. There are always different types of microorganisms in the fodder, which contain cereal crops. Such saprophytic microflora can multiply under normal conditions and it leads to lower level of nutritional value of the feed. However, the feed can also include pathogenic microorganisms, coliform bacillus bacteria, which aggravate the sanitary condition of the product. The feed Storage at low temperature and humidity can extend their safekeeping term. At low temperatures, neither the microorganisms nor insects cannot progress very fast, and also may less intensively occur in animal feed and various oxidation processes [1].

During the feed storage, its stability depends on the quality and quantity of ingredients included in the recipe. Accordingly, the terms of storage for various types of products were set out in the feed industry. In industrial complexes mixed cattle feed (also for poultry) can be stored for 1 month from the production, the shelf life of other feeds can be 2 months from the production time.

The development of microflora during storage of the product is always accompanied by a reduction of quality because of decomposition of organic substances and accumulation of waste products of microorganisms, which are dangerous for agricultural animals, poultry and fish.

The extrusion process can contribute to get high sterile product with low mass part of moisture, which gives an opportunity to extend its shelf life. At the same time, the advanced specific surface and increased hygroscopic cause negative effect of products' quality, which changes during the storage [3, 16].

Recently, the industry more and more attention paid to the use of oil crops not only for vegetable oils, but also as a source of complete protein, biologically active substances and various macro- and micronutrients.

Traditionally, protein and oil crops used to produce vegetable fats, which are then used for food and technical purposes. By-products after the removal of fat – cake and meal, are widely used for animal feeding and receiving various protein concentrate [6].

Recently, more attention is paid to the use of whole seeds of these crops as for food and for fodder purposes, particularly the use of such non-traditional crops as flax.

Recent studies increasingly reveal the full range of properties of flax seeds, biological value which determines not only the content and composition of fat, but also a significant content of protein, vitamins, enzymes, mucus, organic acids and minerals [15].

Production of feed for farm animals that could compete in quality with foreign analogues requires improvement of existing and development of new highly efficient

technologies. Today in Ukraine there is no perfect technology for the production of animal feed farm animals using flax seed, so to ensure the quality and competitiveness of animal feed products is necessary to introduce the latest technological operations and advanced methods of processing raw materials [7].

## Materials and methods

During the experiments, the changes of microbiological status of extruded grain mixtures were explored during the storage by the following indicators: total number of microbial cells (MAFS), the presence of pathogens especially Salmonella, the presence colon bacillus bacteria (coliforms, CFU/g), the presence of anaerobes and aerobes. We used the grain of wheat, corn, and flax extract, based on wheat to create extruded batches of grain mixtures. Such mixtures had the following percentage composition:

- blend 1 – wheat – 45%:corn – 45%: flax extract, which is based on serum – 10%;
- blend 2 – wheat – 45%:corn – 40%: flax extract based serum – 15%;
- blend 3 – wheat – 40% corn 45%: flax extract based serum – 15%;
- blend 4 – wheat – 40% corn – 40%: flax extract based serum – 20%;

Flax extract, which is based on wheat, was obtained by extraction in pulsating dispersers, with the active diaphragm. There is a powerful cavitational effect on the dispersed phase in these apparatus that provides intensive secretion of bioactive substances in the volume of the solvent. There are some effectual technologies, which are used in various industries, which are based on application of pulsator aggregates of discrete-pulse energy input (DVI) with periodic change of pressure in the working chamber.

This method shows the new approach of heat and mass transfer intensification and hydro-mechanical processes in dispersion systems [5].

The influence of cavitation effects pulsating dispersant extraction was carried out at linseed, focusing on indicators of physical and chemical properties and biological value. For this purpose, flax seed milled 500 g with the addition of 3 liters of serum. Percolation process took 2 hours at 30 °C. The extraction was carried out for 5 and 10 minutes. Research data extraction representation in Table 1.

**Table 1**

**Physic-chemical characteristics of extracted liquid solution**

| Parameter | Extracted liquid solution | |
|---|---|---|
| | Processing time, s | |
| | 300 | 600 |
| Total mass fraction of dry substances, % | 8,4 | 7,3 |
| Raw protein, % | 25,1 | 25,9 |
| acidity, degree | 4,4 | 4,0 |
| Starch, % | 11,4 | 11,0 |

So, after the researches we can say the following:

The number of dry substances is reduced in the sample solutions of milled flax, based on wheat according to different processing time in the apparatus, which shows better solubility under the pressure. Analyzing such indicators like acidity and starch, we noticed that they are inversely proportional to the extraction period in the apparatus and the protein's amount can increase in directly proportion to the extraction time reaches its maximum when the period of extraction continues at least 10 min.

It is known that the heat resistance of microorganisms depends on the properties of the environment, which is of floral mixture, which is heated, namely the quantity and condition of protein, fat, pH. Bacteriological determination of thermal death of microorganisms – that's when they lose the ability to restore their vital functions. The maximum temperature resistance of microorganisms is in a small range from pH 6.0 to 7.0 and beyond this range it decreases sharp. In our case, multi-factor mechanisms DIV leads to a significant increase in pH from 4.7 in the starting mixture to a pH of 6.5 to 6.8 after processing in a rotary pulse apparatus (RIA).

Khanoll A. M. and Riman G. I. set a staement that "Bacteria are consider dead if they lost the ability to refurbish even when they have the most optimal conditions for their existence. "When monofactorial heat cells get damaged, which in favorable conditions (environments) restore their vital activity, and in other conditions it leads to cell death [9, 16].

After the resumption of injury, which was caused by warming up and cells can multiply as cells, that did not receive the action of heat. It means that, the restoration of sub lethally thermal damage is a process, which takes place under specific conditions in which the ability to reproduce and normal structure and function of cells can regenerate. The death of the microorganisms from the action of heat depends on the duration of the process, the condition and environment's properties and only in the absence of resistance; the curve of destruction of microorganisms can be a line [13].

After the thermal process a certain number of microorganisms that are capable of restoration and further reproduction can survive. G. Lembke explained it in such way, that the action of energy the hydrogen bonds can be damaged in the cell, but they can be renewed due to the long-wave light or substances that carry an electrical charge and are able to contact with hydrogen [4].

Therefore, preliminary processing in the RIA before the extrusion, where there are mechanisms of discrete-pulse energy input, provides:

- the maximum reduction of vegetative bacteria;
- reduction of spore bacteria;
- reduction of mold and yeast.

This result is achieved at low temperature pasteurization, namely – 72–76 °C with an exposure of 2...3 minutes. Also keep in mind that the processing of RIA devices by multi-action mechanisms DIPE increases temperature 30–35 °C, which significantly affects the energy efficiency technology.

## Results and discussion

Determination of the qualitative and quantitative content of microorganisms in extruded feed mixtures was carried out according to the rules for animal feed [4, 5].

To control the qualitative and quantitative composition of microorganisms were taken some samples from fresh prepared extruded mixtures, which consisted of wheat-corn-flaxseed extract, based on whey. Test samples were set in cloth bags and stored for 2 months at 0 °C (refrigerator), 20 °C (thermostat) and a relative humidity of 45%.

The moisture of the grain mixtures before the extrusion in the samples was in the range of 16.8–17.6%. The moisture content after extrusion ranged from 13.3 to 13.5%, and during the storage period it has not changed under the conditions: temperature 0 °C, relative humidity 45%. But at the temperature of +20 °C and relative humidity of 45% was recorded some changes of this index. The data changes of humidity (under conditions: temperature + 20 °C and relative humidity 45%) are shown in Figure 1.

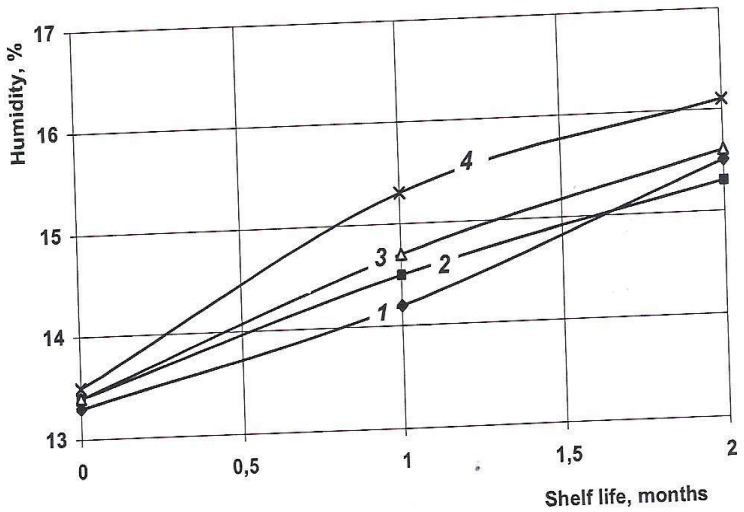


**Figure 1. Change of moisture during storage prototypes:**
1 – blend 1; 2 – blend 2; 3 – blend 3; 4 – blend 4;

As you can see from the figure, the change in humidity of explored samples under the normal conditions, increases during the storage and at the end of the second month of storage is 15.3 to 16.1%.

Comparing the conditions and period of storage, it is possible to say that changes in the moisture content of the extruded feed mixture largely depends on the storage temperature, and not on the amount of introduced flax extract whey in the mixture.

From the above words it follows that the moisture absorption by samples of a research largely depends on the storage conditions. The results of experiments regarding the level of microbiological contamination of extruded feed mixtures during storage are presented in Table 2.

Analyzing the data of Table 2 for all samples it shows that, as at the beginning, so at the end of storage of extruded feed mixtures, characterized by rather low level of microbiological contamination. During the exploring we did not detecte the following groups of microorganisms, such as colon bacillus bacteria and pathogens. The total number of mesophilic aerobic and facultative anaerobic microorganisms (SAFS) in all samples of mixtures is in the acceptable range (not more than 5·105 CFU/g) [10–16]. In experiments of the microflora of the mixtures it was found that each sample is characterized by its particular special microflora (Table 3).

Analyzing the colonies of mesophilic aerobic microorganisms on beef-extract agar set that they are characterized by large, small and medium sizes, white and yellow color, smooth and jagged edges. The main part of the microorganisms are coccal bacteria, whose cells are located one by one or in small groups (clusters). The main morphotypes of bacteria isolated from grain extrudates, are aerobic bacteria.

Table 2

**Change microbiological extruded feed mixtures during storage**

| The title of extruded product | The storage time (days) | The number of mesophilic aerobic and facultative anaerobic microorganisms | Colon bacillus bacteria, | Pathogens | Anaerobics |
|---|---|---|---|---|---|
| Temperature +20° C | | | | | |
| Blend N 1 | 0 | < 10 | no | no | no |
| | 15 | $2 \cdot 10$ | no | no | no |
| | 30 | $9 \cdot 10$ | no | no | no |
| | 45 | $1 \cdot 10^2$ | no | no | no |
| | 60 | $1{,}7 \cdot 10^2$ | no | no | no |
| Blend N 2 | 0 | < 10 | no | no | no |
| | 15 | $3 \cdot 10$ | no | no | no |
| | 30 | $9 \cdot 10$ | no | no | no |
| | 45 | $1{,}3 \cdot 10^2$ | no | no | no |
| | 60 | $2{,}6 \cdot 10^2$ | no | no | no |
| Blend N 3 | 0 | < 10 | no | no | no |
| | 15 | $5 \cdot 10$ | no | no | no |
| | 30 | $1{,}1 \cdot 10^2$ | no | no | no |
| | 45 | $1{,}7 \cdot 10^2$ | no | no | no |
| | 60 | $3 \cdot 10^2$ | no | no | no |
| Blend N 4 | 0 | < 10 | no | no | no |
| | 15 | $9 \cdot 10$ | no | no | no |
| | 30 | $1{,}8 \cdot 10^2$ | no | no | no |
| | 45 | $3 \cdot 10^2$ | no | no | no |
| | 60 | $5 \cdot 10^2$ | no | no | no |
| Temperature 0 °C | | | | | |
| Blend N 1 | 0 | < 10 | no | no | no |
| | 15 | < 10 | no | no | no |
| | 30 | < 10 | no | no | no |
| | 45 | < 10 | no | no | no |
| | 60 | < 10 | no | no | no |
| Blend N 2 | 0 | < 10 | no | no | no |
| | 15 | < 10 | no | no | no |
| | 30 | < 10 | no | no | no |
| | 45 | < 10 | no | no | no |
| | 60 | < 10 | no | no | no |
| Blend N 3 | 0 | < 10 | no | no | no |
| | 15 | < 10 | no | no | no |
| | 30 | < 10 | no | no | no |
| | 45 | < 10 | no | no | no |
| Blend N 4 | 60 | < 10 | no | no | no |
| | 0 | < 10 | no | no | no |
| | 15 | < 10 | no | no | no |
| | 30 | < 10 | no | no | no |

Table 3

**Characteristics morphotypes of microorganisms isolated from extruded cereal mixes**

| Blends | Attitude to oxygen | Type of collonium | Size of Collonium | Shape of collonium |
|---|---|---|---|---|
| Blend 1 | Aerobics | Round, white, with smooth edges | Small | Coccus, potty, accommodate with small groups |
| Blend 2 | Aerobics | Round, white, with smooth edges | Small | Coccus, potty, accommodate with small groups |
| | Aerobics | Round, white, with jagged edges | Medium | Coccus, potty, accommodate one by one |
| Blend 3 | Aerobics | Round, white, with smooth edges | Small | Coccus, potty, accommodate with small groups |
| | Aerobics | Round, yellow, with smooth edges | Medium | Coccus, potty, accommodate one by one groups |
| Blend 4 | Aerobics | White, with jagged edges | Medium | Coccus, potty, accommodate one by one |
| | Aerobics | White, like amoeba | Big | Coccus, potty, accommodate one by one |

## Conclusions

The results of experiments show that a humidity of prototypes can change and it depends on storage conditions. At 0 °C and a relative humidity of 45% humidity samples does not change during two months of storage, and is in the range of 13.5 to 13.3%. At a temperature of + 20 °C and relative humidity of 45% moisture content of samples changed during storage from 13.3–13.5% to 15.3–16.1%. Extrusion is an effective way of improving the sanitary quality of extruded grain mixtures, because it allows reducing the number of microorganisms. The research of quantitative and qualitative composition of the microflora of extruded grain mix with the addition of flax extract, based on the whey showed that the total microbial number (SAFS) in all the test samples are situated in acceptable limits of not more than 5·105 CFU/g). Indicators of bacillus coli bacteria were not detected. The absence of pathogenic and conditionally pathogenic microorganisms indicates the provision of adequate sanitary and hygienic conditions in the manufacture of extruded blends.

During the storage of experimental batches of extruded grain mixtures for two months at temperature of + 20 °C, there is a slight increase in microbiological parameters and at 0 °C there is no growth of microorganisms.

## Вплив екструдування на мікробіологічні показники кормових сумішей

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**Вступ.** З метою визначення якості кормових сумішей, що містять лляний екстракт на основі сироватки було досліджено мікробіологічні показники кормових сумішей після екструдування різного рецептурного складу під час зберігання.

**Матеріали та методи.** Досліджувалися кормові суміші з зерна пшениці, кукурудзи та лляного екстракту на основі сироватки у різній відсотковій кількості. Лляний екстракт отримували шляхом екстракції в пульсаційних диспергаторах з активною діафрагмою Суміш змішували та екструдували за температури – 110–120 °С, тиску – 2–4 МПа, що дозволяє майже повністю її знезаразити.

Досліджували зміну мікробіологічних показників кормових сумішей під час зберігання. Дослідні зразки поміщали в тканинні мішки і зберігали впродовж 2 місяців за температури 0 °С (холодильник), +20 °С, (термостат) і відносній вологості повітря – 45%.

**Результати та обговорення.** Вологість зернових сумішей до екструдування в зразках знаходилась в межах 16,8 – 17,6%, після екструдування від 13,3 до 13,5%, та на протязі терміну зберігання не змінювалась. За температури + 20 °С і відносній вологості повітря 45% вологість зразків змінюється в процесі зберігання від 13,3…13,5% до 15,3…16,1%.

Зміна вологості екструдованих кормових суміш в значній мірі залежить від температури зберігання, а не від кількості введеного лляного екстракту на основі сироватки до складу суміші.

На початку та в кінці зберігання екструдованих кормових сумішей, характерний досить низький рівень мікробіологічного обсіменіння. Не були виявлені такі групи мікроорганізмів, як бактерії групи кишкової палички (БГКП), патогенні мікроорганізми. Загальна кількість мезофільних аеробних і факультативно анаеробних мікроорганізмів (МАФАМ) у всіх зразках сумішей знаходиться в допустимих межах (не більше $5 \cdot 10^5$ КУО/г).

Аналіз колоній мезофільних аеробних мікроорганізмів на м'ясопептинному агарі виявив, що вони характеризуються великими, малими і середніми розмірами, білим і жовтим забарвленням, рівними і нерівними краями. Основна частина мікроорганізмів є кокові бактерії, клітини яких розміщуються поодиноко або скупченнями. Основними морфотипами бактерій, виділених із кормових екструдатів, є аеробні бактерії.

**Висновок.** Рекомендується використовувати процес екструдування як ефективний спосіб підвищення санітарної якості екструдованих кормових сумішей, оскільки він дозволяє отримати майже стерильний продукт.

**Ключові слова:** *корм, льон, екстракт, мікробіологія, коки.*