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Lactic Acid Bacteria Fermentation for Detoxification of Castor Bean Meal and Processing of Novel Protein Feeds Supplement

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Abstract— ***The study presents the details of microbial liquid culture method for eliminating toxicity of castor meal and producing concentrated protein product. The research was focused on lactic acid bacteria, important in food industry, agriculture and environment. The conditions of hot alkaline protein extraction from meal were optimized and found to be 25% meal in 0.4% alkali solution at 90°C for 30 min. Streptomyces thermophilicus, Str. diacetilactis and Lactobacillus acidophilus were chosen from the numerous laboratory collections as the most prominent starters for fermentation of alkaline protein extract contained toxic antinutritional compounds. The triple microbial formulation of these cultures was found to be the most effective in rapid development of stable protein curd with adequate nutrition value and protein quality. The, extremely toxic ricin was completely eliminated and the fermented product has good nutritional profile with high level of water-soluble protein and essential amino acids. It was concluded that fermented product may be recommended as a promising acceptable protein source in feed production.***

Index Terms— **Castor meal, Microbial fermentation, Protein Feeds Supplements.**

I. INTRODUCTION

Castor (*Ricinus communis*) is an important oilseed crop with many industrial and agricultural applications. It is cultivated mainly in tropical climate, and the main producers are India, Brazil and China. Global production of castor seeds is reached 2.77 million metric tons in 2011[1]. Typically, castor oil is extracted from seeds by hot- or cold-pressing procedures that yield castor meal. Since castor seeds contain roughly about 50% of oil and 50% of residual meal, a large tonnage of protein-rich castor seed meal is generated each year in the world. Castor meal is applied as organic nitrogenous fertilizers [2], soil conditioners [3], and pesticides for nematodes and insects [4], and used for the production of industrial enzymes, antibiotics, biopesticides, vitamins and other biochemicals. Due to their rich protein content it was also used as feed supplement for sheep [5]), cattle [6] and poultry [7].

The main problem in castor meal application as feed component is the high content of extremely toxic components, namely ricinine, a toxic alkaloid; ricin, a highly potent ribosome-inactivating protein toxin; a tetrameric protein that agglutinates mammalian erythrocytes and three effective allergenic proteins [8]. Various methods including physical, chemical and biological treatments have been employed to detoxify the castor meal [9], but the detoxification process is not always effective [10]. For these reasons, currently castor meal not used commercially for animal feed in the Europe and USA and is most often used as a natural fertilizer since it is rich in nitrogen. In this study, we report a novel microbial mediated approach for detoxification of castor meal and producing safe, protein products with high nutritional fodder and rich in essential nonreplaceable amino acids.

II. MATERIALS AND METHODS

Samples of castor bean meal were donated by the vegetable oil- extraction plant “Belorechenskiy”, Krasnodar region, Russia. For the characterization of the raw material, the meal was submitted to classical chemical analyses and analyzed for pH, protein, lipids, crude fiber, ash and moisture content. For protein extraction the meal was ground and proteins were extracted by solubilization in an alkaline solution under different concentration of alkali (1, 2, 3, 4 and 5% w/w), temperature (28, 60 and 90ºC), concentration of the meal in the extraction solution (10, 15 and 25%), agitation speed (400 and 600 rpm) and time of extraction (10, 20, 30, 60 min). The protein extracts (supernatants) were separated from the solid residues by centrifugation (4000 rpm/20 minutes) and analyzed for protein, dry matter and ash. Amino acids content of lyophilized proteins was analyzed using SYKAM Amino Acid Analyzer. The analysis of ricin in was done using Waters 616/626 HPLC.

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The crude protein extracts were applied for laboratory fermentation with monoculture or complex formulation of lactic acid bacteria (LAB). The *Lactobacillus* and *Streptococcus* used as starter cultures were isolated in our laboratory from traditional fermented milk products and maintained on nutrient agar slants or skim milk in the refrigerator prior to use. The laboratory fermentation in static conditions was done at 30^{o}C for 72 h using the method described in [11]. LAB cultures were incubated aerobically for 48 h at 37°C on MRS agar, Oxoid or sterilized skim milk enriched with 0.1% yeast extract. Cells from the broth culture were harvested and washed three times with 0.1 potassium phosphate buffer by centrifugation at 14000 rpm for 5 min. The cells were then aseptically re-suspended and cell suspension was standardized using a spectrophotometer (at 600 nm 1.0 mL of the suspension contained about 3.0 × 10^9 cells). The starter inoculums rate was 3% v/v. The data were analyzed statistically by analysis of variance and differences among the significant treatments were determined by least significant difference (LSD) test. All data presented were the mean of three different plots, i.e., $n = 3$.

III. RESULTS AND DISCUSSION

It was shown that tested raw castor bean meal was rich in nutrients and contains all necessary basic components required for growth of LAB as an energy source, including 2% w/w of sugars (sucrose, raffinose, and glucose) and 2.5% w/w of lipids. Amino acids and peptides may be utilized after enzymatic hydrolysis by proteases.
Firstly we have developed the effective protocol of alkaline extraction of protein from dry coarse meal powder. Experimental data showed that the growth of alkalinity and temperature significantly ($P< 0.01$) increased the effectiveness of protein extraction (Fig.1). The effect of agitation speed, time and extraction ratio was less clear, although the quantity of proteins increased.

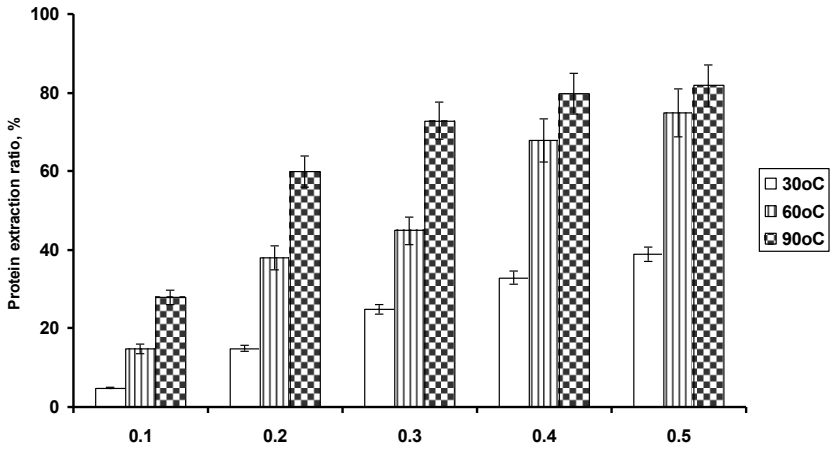

Fig.1: Effect of alkali content and temperature on effectiveness of protein extraction from castor meal. Extraction time 30 min, agitation speed 600 rpm, and meal concentration 25%.

Fermentation is one of the oldest ways of food processing which is carried out by microorganisms and their enzymes in order to achieve desirable quality of products. During the process, microorganisms transform food components; enhance their palatability, increases protein value, vitamin content and mineral levels. It not only, improves nutritional value, but also reduces antinutritional compounds. Currently there are very scarce information about microbial fermentation of castor seeds, meal and their byproducts. In African countries product from fermented castor oil seeds, called "*ogiri*" forms an important part of the diet. It was found that predominant microorganism involved in this fermentation process was *Bacillus subtilis*, and starter monoculture was proposed as to standardize the product production [12].

We proposed the original fermentation technology of alkali-extracted castor meal by lactic acid bacteria (LAB).
The LAB genera generally present in fermented milk products are *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Enterococcus* and *Pediococcus* [13]. LAB initiate the process of fermentation whereby carbohydrates in the milk are oxidized into predominantly lactic acid, but alcohol, carbon dioxide and several other compounds can also be produced depending on the LAB strains present. These microbes may also produce other organic acids such as acetic, propionic, formic and butyric acids, as well as enzymes, bacteriocins, aroma

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compounds and exopolysaccharides [14]. As a result, raw materials are converted to a safe product with a reduced pH and unique sensory characteristics.

We have tested a large collection of LAB pure cultures for their ability to grow on meal extract and form protein curd. Two *Streptomyces* strains (*Str. thermophilicus* and *Str.diacetilactis*) and *Lactobacillus acidophilus* were found to be the most prominent starters and were applied for further investigations.

The effective starter bacteria for feed production from castor meal shell comply with the some special requirements. Firstly, the process of fermentation must be as quick as possible and desirable growth conditions for the starter bacteria (temperature, pH, aeration, etc) must be moderate, not extreme. Secondly, the starter strains must produce the desired final product with the sensory characteristics correspondent to the dietary habits and preferences of the target consumer communities. Thirdly, the starter strain(s) must be able to reduce toxicological risks of foods if the raw product contains toxic chemical compounds.

It was shown, that application of triple microbial formulation (*Str.thermophilus*: *Str.diacetilactis*: *L.acidophilus* = 2:2:1 v/v) was the most effective and significantly (1.5- 2.5 times) decreased the time of curd formation (Fig.2). As a result, only 4 hours was necessary to get fermented protein product (FPP) by active LAB formulation. LAB growth required complex and rich media, containing organic nitrogen sources (peptides), carbon sources, vitamins and minerals. These nutrients should be supplied at optimal concentrations.

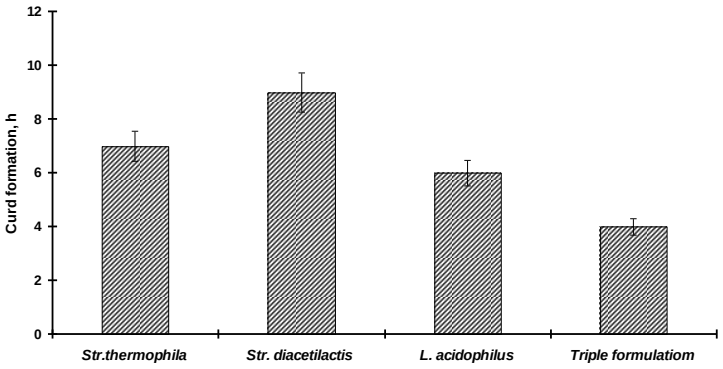

Fig 2: The time of FPP formation at fermentation process of alkaline protein extract from castor meal by monocultures and triple formulation of LAB.

The end-products produced by LAB mainly include lactic acid and the pH values of the fermented samples decreased. The initial pH of protein medium was 11-12, and after 3 hours for monocultures and within 1.5-2 hours for microbial formulation the pH decreased and reached 7-5. The acidification of protein medium is the factor of protein coagulation and formation of homogeneous dense curd. The growth and fermentation activity at strong alkaline medium (pH 11-12) is novel, not described characteristic of LAB.

In order to get the best sensory characteristics of protein product we have multi-factorial designed experiments. We have compared protein samples extracted in different conditions and evaluated some physicochemical and organoleptic features of fermented protein product (FPP) (Table 1).

It was found that the optimal characteristics of FPP were achieved when the extraction duration was 25-30 minutes at 90-96^{o}C and mixing ratio 1:8. The use of low temperature and short extraction time resulted in increase of fermentation time and degradation of organoleptic characteristics.

It is very important, that no ricin, the main toxic compound, was found in all samples of FPP. So, the application of LAB fermentation is the technique of complete detoxification of castor meal. Current application of castor meal as feed product is restricted and not used commercially in the Europe and USA due to toxicity. In the 1960's cattle feed product containing detoxified castor meal was sold in Brazil and no negative effect on animal's health was found [15]. Conversely the deaths of dogs were reported after ingestion of product contained 10% of castor meal [10]. Ruminants appear to possess greater tolerance for castor meal in their diets than monogastric mammals [16].

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There are numerous effective detoxification methods including autoclaving, boiling, treatment with sodium or calcium hydroxide [17] and alkaline methanol [18]. The advantage of our approach is simplicity, economical efficiency and environmental friendliness. It was found that the optimal characteristics of FPP were achieved when the extraction duration was 25-30 minutes at 90-96^{o}C and mixing ratio 1:4. The use of low temperature and short extraction time resulted in increase of fermentation time and degradation of organoleptic characteristics.

Table 1: Comparative characteristics of castor meal, protein extract and fermented protein products.

Sample	pH	Fermentation time, h	Organoleptic estimates	Ricin content, %
Castor meal	No	No	Coarse gray-brown powder	1.4
Protein extract	10.5-12	No	Creamy liquid	0.03 – 0.01
FPP from protein samples with different extraction conditions				
A. Temperature of extraction, ^{o}C				
26-28				
55-60	6.5	24-25	Light creamy loose curd with whey	0
90-96	7.0	10-12	Light creamy dense curd with whey	0
	6.5	4.5-5	Light creamy dense curd	0
B. Extraction time, min				
10-15				
25-30				
40-45	6.5	8.0-9.0	Light creamy loose curd	0
	6.0	4.5-5.0	Light creamy dense curd	0
	6.0	4.5-5.0	Light creamy dense curd with whey	0
C. Ratio of meal and alkali solution				
1:10	6.5	8.0-9.0	Light brown loose curd	0
1:8	6.2	4.5-5.0	Light creamy dense curd	0
1:4	6.0	5.0-5.5	Light creamy dense curd	0

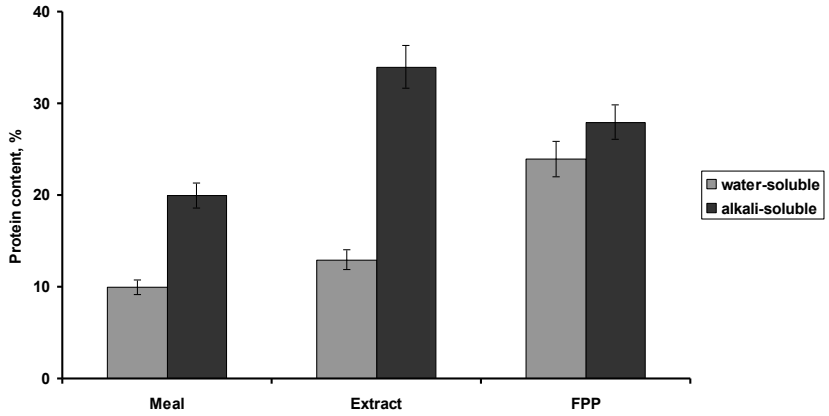

Fig 3: Nutritional protein quality of FPP.

Microbial fermentation significantly improved nutrition value of protein product as compare with protein extract form meal. Firstly, the water-soluble part of protein, the most valuable in the forage protein fractions, was significantly increased in FPP (Fig. 3). Proteins are organic compounds, which are necessary to grow new tissues and to repair old tissues in an animal. Since most feedstuffs are low in proteins, protein supplements may be necessary.

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Other very important characteristics are the high content of indispensable amino acids (Table 2). Amino acids are the building blocks of proteins and are classified as either essential or nonessential. Most animals can synthesize the nonessential amino acids. However, the essential amino acids must be supplied in the diets of nonruminant animals. Monogastric animals are unable to synthesize amino acids and thus should have their diets supplemented with proteins containing the 10 essential amino acids. Ruminants are capable of synthesizing all amino acids by microbial action in the rumen.

Table 2: The amino acid composition in protein feed product received from castor meal.

Amino acid	Content in FPP, mg g^{-1}
Aspartic	115.0
Threonine*	31.7
Serine	60.0
Glutamic	296.4
Proline	51.5
Glicyne	43.5
Alanine	48.4
Valine*	57.1
Methionine*	21.1
Isoleucine*	44.0
Leucine*	64.0
Tyrosine	7.7
Phenylalanine*	42.3
Histidine*	16.4
Lysin*	26.8
Arginine	6.82
Sum of essential amino acids*	***267.9***

IV. SUMMARY AND CONCLUSION

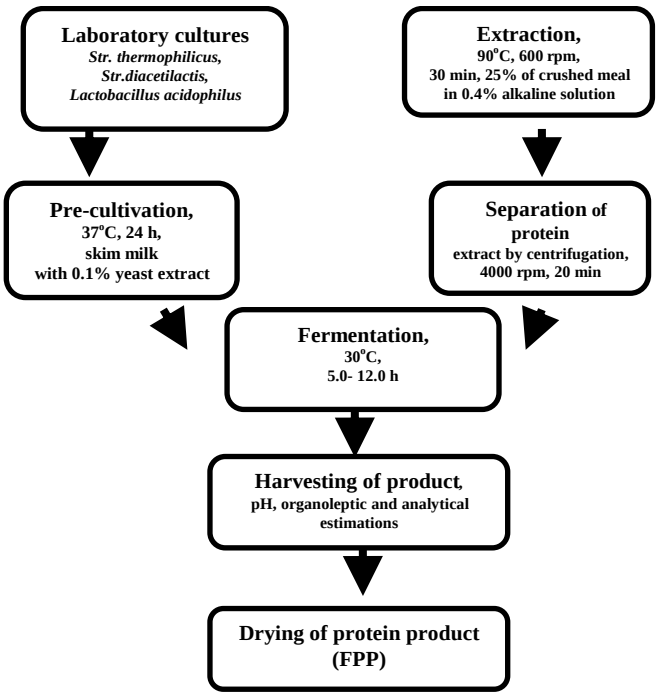

Fig 4: Flow diagram for fermentation production of protein product from castor meal.

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Currently the growing world population places a demand on agricultural and food industries to increase the production of safe and nutritious foods for people and feed for livestock. Plant and animal agricultural byproducts derived after extraction of a high value component can provide lower cost sources, which is particularly important for animal feed. The world demand for additional protein supplies has encouraged studies and exploitation of various inedible protein-rich byproducts. One of these is castor bean meal which contains about 40% of crude protein but has found only very limited use as a feedstuff or food because of the presence of three potent toxicants: Ricin, castor allergen and ricinine. In the present study, we investigated the effectiveness of novel biotechnological approach based on acid lactic bacteria fermentation to detoxify castor meal and to improve its protein quality through supplementation with the essential amino acids. The scheme of process in described on Fig.4.The results indicate that fermented protein product has good nutritional profile with high level of protein and essential amino acids and is free of toxic components.

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