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INNOVATIVE METHODS FOR IMPROVING THE MICROBIOLOGICAL SAFETY OF GERMINATED BROAD BEAN SEEDS

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Abstract: The microbiological safety of germinated broad bean seeds is crucial for their utilization in nutritious food products. This study aimed to investigate effective methods of ensuring the safety of germinated seeds using bio-activated seed and grain. Our findings indicate that short-term hydrothermal treatment and seed shell removal are effective in improving the microbiological purity of bio-activated beans. Storing sprouted seeds in an insoluble form at +6°C in polyethylene bags for up to four days was also found to ensure their safety during storage. This study suggests a safer and secure method of deriving germinated raw material and semi-finished goods, opening functional usage of derived products.

Keywords: Broad bean seeds, germination in peeled form, microbiological indicators, hydrothermal treatment, storage.

Introduction

Germinated cereal products, likewise natural, are being used in health-improving diets [1-3]. Research conducted last years in this direction for the inventions of the products of functional purpose have obtained huge scales [4-8].

Based on it, our research with broad bean seeds, which are called "pakhla" in the folklore like traditional protein-containing raw material, widely used in cooking, in order to get germinated composition and food products from it deserves attention [9-13].

It is well known, that wheat and other cereals are germinated with the temperature of 20-40 ºC [14-16]. Herewith, active growth of foreign microflora was observed which degrades the safety of germinated raw material, as germinated grain contains in average 42-44% moisture, which is a favorable environment for development of microorganisms [17,18].

Usually for decreasing the amount of pathogenic microorganisms in the process of germination, a number of ways is used – mixing in the water simultaneously soaking antimicrobial agents, processing raw material by them, or reduction of the germination time and other ways. In latter (reduction of the germination time), it is achieved at the expense of exercitation of enzyme preparations, destroying membranes of cereal, in which main ingredients are cellulose and some hard-hydrolysable polysaccharides (lignin, pentosane, pectin substances, protopectin and etc.).

In spite of a certain significance of indicated components of the membrane, they prevent acceleration of biochemical reactions, contain foreign microorganisms and decrease the nutrition value in the process of germination. Most of the bean seeds in the membrane contain contemporary anti-nutritional substances (oligosaccharides, antitrypsin and antipepsin enzymes and etc.), which further decrease food-producing values and physiological significance of the processed cereal.

In a word, despite of the popularity and high nutrition values, derived products in terms of germinated cereal and their germinates, primarily leguminosaes, possess major deficiencies – quick mold formation, propensity for medical problem with potato diseases and others. Especially this type of the product is most of the time primarily offered to the usage in raw form by way of additives for salads, porridges, soups, and others. With that, washing them before using does not entirely provide the level of their microbiological safety.

All this require academic specialists to develop safer and secure methods of derivation of germinated raw material and semifinished goods for nutrition ration.

Proposed modified method by us of having the germinated broad bean seeds, in our view, meets above indicated requirements and makes impossible the promotion of the level of microbial contamination of the germinated production. As in the process of germination of the latter both at the start of preparation of the seeds and their preliminary soaking, results in significant decrease of microbiological filth of the raw material, due to smooth regulation of the preliminary short hydrothermal treatment for removing the most infected details of the beans – seed's shell. With that, the process of preparations of the germinated samples, promotion of their appearance and improvement of nutritive value of the ready production increases.

This article indicates the results of the exploration of microbiological safety of the preliminary peeled and bio activated (germinated) broad bean seeds from the Windsor variety, growing in south regions of Azerbaijan Republic, whose germination was implemented by us with former suggested method [19]. Whereby, used raw materials in content of toxiferous elements (mg/kg), pesticides (mg/ kg) and mycotoxins (mg/kg) were in line with demands of current standards, in other words were safe for germination.

Thus, in order to decrease the microbial contamination of germinated seeds of broad beans and the time of its germination, increase the activity of endoenzymes, increase the production of the germinates in a developed process, short-lived special wetheat treatment and removing bran covering, before soaking the raw material, was applied. After that the peeled seeds (seed lobe) were soaked in drinkable municipal water at the temperature of 20º±4ºC (hydro modulus composed 1:10) during 21 hours, then were germinated during 72 hours. Every 12 hours the amount of the germinated seeds to their total number were written down (Figure 1).

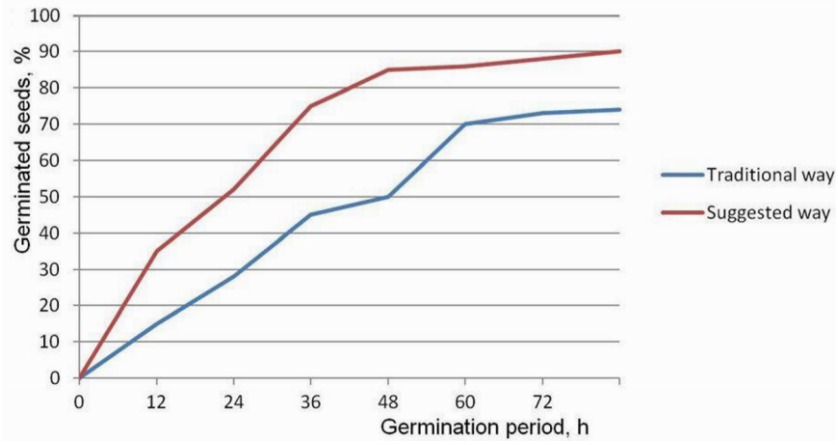

Figure 1: The dynamics of sprouting of the broad bean seeds depending on germination method 1.Traditional method (natural unpeeled seeds) 2.Suggested method (peeled seeds)

External appearance of the seeds of broad beans before and after germination, depending on method of preproduction of the raw material, is shown in Figure 2.

Figure 2: External appearance of the seeds of broad beans before and after germination depending on method of preproduction of the raw material **(A)** natural seeds; **(B)** Peeled seeds; **(C)** Germinated by traditional way It should be noticed, that in the process of germination of peeled bean samples by suggested method, they produce double sprouts. However, at natural unpeeled samples it is hardly in evidence, in other words they possess single sprouts. Besides, the process of germination happens much more slowly in comparison to traditional way of germination. **Results and Discussion**

The results of research on changes in morphological factors and appearance of the seeds in the process of germination are shown in the Table 1.

Kind, used for germination of seeds	**Average dimensions of dry bean seeds, sm**		**Middle dimensions of alone and binominal sprouts after germination, sm**		**Middle mass of unpeeled and peeled germinated seeds with germinants (g)**		**Alternation of mass of the seeds after germination, (%)**
	Length	**Width**	**1-st**	**2-nd**	**Before germination**	**After germination**	
With a husk (unpeeled)	3,0	2,0	1,9	-	2,7	7,3	267

Without a husk (peeled)	2,8	1,8	2,5	1,6	3,0	11,5	380

Table 1: Alternation of morphological factors and appearance of the broad bean seeds in the process of germination

As seen from Table 1 and Figure 1, preproduction and removal of shell, simultaneously improving the arising sprouts, increase the dynamic of their germination. The microbiological contamination of broad bean seeds, germinated by traditional and suggested way, was determined by well-knowing methods [20-22].

The results on determination of changes in microbiological factors of broad bean seeds in the process of germination are shown in the Table 2. As seen in the Table 2, natural seeds in the process of preparation for germination possess twice as large contamination, than the seeds removed from shell by applying preliminary hydrothermal treatment. The same is observed in the finished germinated samples.

Taking into account the high quality and quantitative factors of germinated broad bean seeds by suggested technology and perspective of their usage for deriving functional nutritional products, it is considered to be desirable to further research the condition of microbial contamination of germinated samples in the process of storage, as this products have a short storage time in spite of popularity.

No	**The technology of germination**	**Microbiological attributes afloat seeds, CFU, g**			
		Mesophilic aerobic and facultative anaerobic microorganisms	**CB in 0,1 g seeds**	**Yeast**	**Fungus**
I	Germination of natural unpeeled seeds:				
	-seeds after soaking	$6{,}0 \cdot 10^5$	is absent	not detected	not detected
	-seeds after germination	$1{,}6 \cdot 10^{12}$	Elicited	$3{,}0 \cdot 10^3$	30
II	Germination of peeled seeds:				
	-seed lobes after soaking	$3{,}0 \cdot 10^2$	is absent	not detected	not detected
	-seed lobes after germination	$2{,}5 \cdot 10^6$	is absent	< 15	< 15

Table 2: Microbiological attributes unpeeled and peeled broad bean seeds in the process of germination Thus, developed method of broad bean seeds germination in a peeled form on suggested technology, provides their microbiological stability. We are in opinion that, it occurs due to positive effect of preliminary hydrothermal treatment and soaking of seeds without shell in microflora condition in the process of their germination.

For this reason, in order to increase the drawdown period of germinates (including seed lobe) made from broad bean seeds and for prevention of their recontamination with microorganisms, germinated samples were dried on the air, kept in polythene bag weighing of 100-150g in the refrigerator at +6 ^{0}C till 5 days after washing. Research have shown that, the safety preservation of germinated seeds in polythene bags is provided up to four days, as after five days the bacterial load growths above accepted level of safety [23]. The results are shown in the Table 3.

Indices	**Existence of microorganisms upon storage**		
	2 days	4 days	6 days

MAFAM, CFU/g	$3,0•10^5$	$4,5•10^5$	$5,0•10^8$
Fungus, CFU/g	not observed	not observed	traces
Yeast, CFU/g	not observed	not observed	traces
colibacillus bacteria, in 0,1 g is not allowed	is lacking	is lacking	is lacking

Table 3: The alternation of microbiologic indices of germinated seeds upon storage in polythene bags in the freeze (+6 ºC)

Hence, the conducted research allow to draw the conclusion that, the usage of directed regime of bean seeds germination in peeled form allows not only to speed up the time for germination, but also to derive seed mass with the least microbial contamination.

CONCLUSIONS

1. Short-term moisture-thermal processing of seeds of broad beans for peeling and anti-nutritional factors reducing purposes provides a simultaneous improvement in the yield of seeds and their microbiological parameters during germination.
2. Removal of the fruit shell of broad bean seeds by moisture-thermal processing two times reduces the microbiological seeding of the initial seeds.
3. The storage technique of sprouted seeds of broad beans obtained by the proposed method, in polyethylene packaging at a temperature of + 6^0C, ensures the preservation of their safety up to 4 days.

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