The effects of ionic radiation on microorganisms in gluten

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Abstract

Gluten from corn, microbiologically unsuitable for food industry because of the presence of sulfate reducing *clostridia*, was used in this study. Ionic radiation doses of 4, 6, 8 and 10 kGy were used for radicidation of gluten The aim of this study is to set ionic radiation dose for getting microbiologically proper product and to confirm chemical characteristics of gluten after sterilization. Protein content in gluten decreased with the increase of ionic radiation dose, and so did the contents of xanthophyll and carotin, except a noticeable increase in the 10kGy treatment

Introduction

Mechanisms of ionic radiation over microorganisms are based on ionization of molecules, which is not specific In DNA this radiation is both direct and indirect. In indirect reaction, DNA is interacted with H and OH radicals made from water radiolysis. Radicals are released from DNA and the result of this is the forming of monophosphates of purine and pirimidine bases. Direct effect of ionic radiation over a cell is more important than indirect one, because sugar phosphates of DNA are affected and thus stops replication of DNA and causes the death of microorganisms. In irradiated microorganisms, mutual relations of systems for reparation and lethal damages appear The result of this relation can cause the death of a cell. Indirect effects of irradiation on microorganisms can be minimized by freezing, which reduces the migration of released radicals.

There are a lot of reasons, which cause the destruction of microorganisms They include:

The kind of microorganisms
The number of microorganisms
Environment
Temperature
Gas in which microorganism exists
The activity of water (aw values)

All these reasons are very important. It is especially important to know what kind of microorganism is concerned, and besides this, its age, the environment in which this microorganism existed before ionic radiation and stored after the treatment.

Microorganisms are very sensitive when they are ionic radiated. Slow bacteria are very resistant when they are exposed to ionic radiation, gram negative bacteria are very sensitive, so do yeasts and molds. *Micrococcus radiodurans* are very resistant, and *Clostridium botulium* is also resistant to radiation and differs to that of *Cl. sporogens*, which is resistant only when it is effected by thermal treatment.

In the selection of sterilization doses, the number of microorganisms is not important, and what really matters is the kinds of microorganisms and their resistance to radiation.

In food industry, a great attention should be paid to the dosage of radiation in order to keep the value of food products A limited, or very small dose of radiation, can be used with the aim to prolong storage of cut meat, fresh fruit and vegetable, frosty fruit and vegetable By ionic radiation, insects at various phases of their growth in foods can be destroyed Vegetable tissues are very sensitive when they are effected by ionic radiation It is important to note that ionic radiation has economic purpose in food industry.

Today, much research work has been conducted in the field of radicidation, and a lot of information about using ionic radiation for different purposes, is available.

Vidal (1973) described the effects of ionic radiation doses of 70 kGy and 45 kGy over different kind of food, this radiation was used in order to get biologically proper food. In such a way, parasites (Tenia, Trichina), insects, and pathogen microorganisms were destroyed. After ionic radiation, the nutritive value of the treated food had a slight decrease. When frozen and cold products were treated without previous unfrozen, their organoleptic characteristics, color, aroma and nutritive value did not change

Different treatments of sterilization: etilenoxide, microwave and radicidation were used for the sterilization of seasonings. The best effect was achieved by the using of ionic radiation. A dose of 10 kGy was enough for paprika, mash of seasonings and celery seed, and the doses of 14 kGy, 8 kGy and 6 kGy were suitable for onion, garlic and serpolet respectively No organoleptic changes happened (Vaidi, 1970)

WHO, FAO, IAEA gave permission for radiation of a selected kinds of foods by the dose of $\leqslant$10 kGy (1 Megrad)

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Radiation by the dose of 10 kGy per kilogram resulted in heating of 10,000 J. Water effected by this dose of ionic radiation had an increased temperature of 2.4℃ The increases of temperature were 6.8℃ for fat meat, 9.6℃ for floor, 10.5℃ for the aluminum wrapping material and 21℃ for iron wrapping material.

Ionic radiation was used to reduce the number of microorganisms in major ingredients of foods, especially in fish flour and in ground soybean flour Doses of 5 kGy and 10 kGy were used, and the results were compared with that of the control. After radiated, the materials were stored for a period of 90 days and their microbiological characteristics and other parameters were tested. After 5 kGy of radiation, mesophilic bacteria reduced from 10^4 to 10^3 per gram, and the number of molds from 10^3 to 10^1 per gram. Salmonella was completely destroyed by the treatment. The quality was retained after the radiation. The analysis of amino acid structure also showed a good effect of radicidation (Kalman and Somogyi, 1983).

Saint-Lebe (1969) investigated the influence of gamma-rays over technological characteristics of corn starch. In most cases, starch radiated with 3 kGy is enabled to get desired quality, usually without any changes to technological characteristics of the product. Among those modifications, the forming of H_2O_2 is of a great interest for scientists

Sing Ping Lai (1959) thinks that the effects of radiation over gluten can be revealed by the appearance of O_2 and H_2O_2, which play an important role in degradation of gluten.

Material and methods

In this research, gluten was the industry corn product 'IPOK', Zrenanin.

The microbiological quality of gluten was investigated, the process of the corn starch production was controlled too. During our work, we faced the contamination of sulfit-reducing *clostridia*. In some phases, the number of aerobic bacteria and molds, were increasing. From the series of the final gluten, the patterns contaminated with sulfit-reducing *clostridia* were sorted out

The patterns of gluten were inserted in physiological solution by a usual technique, and series dilutions were made.

A usual technique was used (Koch's method) to determine the total number of aerobic mesophilic bacteria in the sample. Sabourad dextrose agar was used to check the total number of molds and yeasts The microbiological results were the average of thirty samples

Other analyses were conducted according to the regulations of microbiological qualities of food.

Chemical analyses

The moisture content: it was determined by drying with sand or on a paper

The determination of starch: the starch content in the above dried material was determined by one of methods used in the analysis of corn

The determination of protein: the Kjeldahl's method was used

Carotin and xantophil were determined by research of material with a mash of hexa-acetone (petroleum-acetone) which was extracted at room temperature, and in the extraction metanolic KOH devastated chlorophyll. Dry extraction was dissolved in petroleter, drifted on the colony of aluminium oxides, where carotenoides separated and at the end, eulates were measured by spectrophotometer.

Amino acid composition of gluten was tested at the division for methods and analyses, the Institute for Microbiological Processes and Applied Chemistry, Faculty of Technology in Novi Sad.

Sterilization by ionic radiation was performed in the Institute for Nuclear Sciences 'Boris Kidric' in Vinca. The following doses of radiation were used: 4, 6, 8 and 10 kGy. The dose of 10 kGy was allowed for the sterilization of food (Sluzbeni list SFRJ 68/84 since 28 December 1984). The radiating source was gamma-rays from ^{60}Co, which decayed and converted into stable isotope Ni.

Table 1. The number of microorganisms in gluten after radiation

Microbiological analysis	Radiation dosage				
	Untreated	4 kGy	6 kGy	8 kGy	10 kGy
Total number of bacteria per gram	110 000	700(0.64%)	200(0.18%)	350 (0.32%)	<10
Escherichia coli in 0.001g	Ø	Ø	Ø	Ø	Ø
Coagulase positive *Staphylococcus* in 0.01g	Ø	Ø	Ø	Ø	Ø
Proteus in 0.001g	Ø	Ø	Ø	Ø	Ø
Sulfate reducing *Clostridium* in 0.01g	+	Ø	Ø	Ø	Ø
Salmonella in 25g	Ø	Ø	Ø	Ø	Ø
yeast in 1gram	< 10	< 10	< 10	< 10	< 10
Molds in 1gram	2500	400	100	< 10	< 10

In our research, gluten with sulfate-reducing clostridia was used, which is microbiologically improper for food industry. No pathogenic bacteria, thermophilic bacteria and yeasts were detected in the samples. The numbers of aerobic bacteria were 110.000/g of samples, and ca 2.500/g of the following molds: *Aspergillus*, *Penicilium*, *Alternaria* and *Mucor*.

In the treatment of 4 kGy, 0.6% of aerobic bacteria and 16% of molds (*Aspergillus*, *Penicilium*) survived, while no sulfate-reducing clostridia was detected. With the dose of 6 kGy, the number of bacteria was 200/g of sample, that is 0.18% of bacteria survived, and only 4% of mold survived (*Aspergillus*, *Penicilium*). After the ionic radiation of 8 kGy, the percentage of survived bacteria was 0.32 % or 350/g. No other microorganisms was detected even after incubation of seven days. The sterilization treatment by ionic radiation of 10 kGy achieved quite sterile product, which was microbiologically stable for three months and remained good quality Thus it can be concluded that the mentioned dose of ionic radiation is efficient for the sterilization of gluten (Table 1)

Chemical analysis of gluten before and after radicidation:

Chemical analysis of gluten enables us to know whether there are any changes in the sample, firstly in the composition of amino acids

Table 2 shows the results of amino acid analysis on gluten (g/100 g of sample) The content of aspartic acid and threonine decreased as the radiation doses increased from 6 kGy to 10 kGy The content of serine remained the same at the dose of 8 kGy, with other two doses it was decreased. The concentration of glutamic acid increased with the increase of ionic sterilazation dose and the maximum dose was 8 kGy The content of prolin was increased with the dose of 10 kGy The decrease of glycine depended on the dose, the least value of glycine was with the dose of 8 kGy. The concentration of alanine remained unchanged in all patterns. The contents of cystine and valine increased with the increase of radiation doses Value of methionine remained unchanged in all patterns. The concentration of isoleucine decreased with the dose of 8 kGy, but with the dose of 10 kGy it showed its initial value. The values of leucine decreased a little with the doses of 6 kGy and 10 kGy. The content of tyrosine decreased with the dose of 6 kGy In g/100g of the pattern, phenylalanine had the least value with the doses of 6 and 8 kGy, but with the dose of 10 kGy it had its initial value The content of lysine increased with the increase of radiation doses and this increase was the most remarkable with the dose of 8 kGy The value of histidine was less in radiated patterns The concentration of ammonia increased suddenly with the dose of 6 kGy, it was stable with the dose of 8 kGy and it decreased, in relation to untreated pattern, with the dose of 10 kGy. The content of arginine changed a little in its radiated patterns.

Table 2. The content of amino acid in radiated gluten (g/100 g of sample).

Amino Acid	Radiation dosage			
	Untreated	6 kGy	8 kGy	10 kGy
Alanine	6.38	6.37	6.44	6.39
Ammonia	1.35	1.52	1.34	1.25
Arginine	2.55	2.42	2.51	2.45
Aspartic acid	4.43	4.41	4.43	4.39
Cystine	0.04	1.01	1.16	1.25
Glutamic acid	16.07	16.11	16.34	16.19
Glycine	2.00	1.95	1.60	1.90
Histidine	1 68	1 49	1.43	1.51
Isoleucine	2.88	2.98	2.40	2.98
Leucine	11.90	11.69	11.96	11.77
Lysine	1.34	1.38	1.42	1.37
Methionine	1.57	1.56	1.57	1.55
Phenylalanine	4.88	4.75	4.72	4.84
Prolin	6.05	6.01	6 05	6.12
Serine	3.49	3.27	3.49	3.23
Threonine	2.36	2.22	2.31	2.22
Tyrosine	4.06	3.77	4.02	3.92
Valine	3.38	3.47	3.47	3.54

The content of protein (dry base) decreased after ionic radiation, but that of the sample treated by 8 kGy remained unchanged. In the patterns of gluten radiated with the doses of 4 and 6 kGy, the content of starch was increased, in other patterns it was approximate to the initial pattern The content of ash was the largest in the pattern sterilized by the dose of 8kGy, in other patterns it was lower than untreated ones. The concentration of oil in initial patterns was 1.92, by the ionic radiation it increased, but it was 1.76 in the pattern treated by the dose of 10 kGy. The content of xanthophyll in mg/kg decreased from 114 (initial pattern) to 37 in the pattern sterilized by the dose of 10 kGy. Carotin in mg/kg decreased in treated patterns differing from the initial ones, except with the dose of 10 kGy when it increased to 28 mg/kg. The other results of chemical

analysis are shown in Table 3.

Table 3. Chemical characteristics of gluten with various doses of radiation.

The kind of analysis	Radiation dosage				
	Untreated	4 kGy	6 kGy	8 kGy	10 kGy
Moisture content(%)	4.0	4.0	4.3	4.3	4.4
Proteins/sm	66.8	65.7	65.2	66.9	65.8
Proteins/tq	64.1	64.2	62.4	64.0	62.9
Starch	14.9	15.4	14.1	15.5	14.6
Ash (%)	1.42	1.11	1.14	1.72	1.33
Oil (%)	1.92	2.15	2.29	2.22	1.76
Xanthophyll (mg/kg)	114	69	97	92	37
Carotin (mg/kg)	22	15	13	12	28

Conclusions

The results of this study affirmed the efficiency of sterilization by ionic radiation.

The number of aerobic bacteria and molds in gluten decreased after ionic radiation. The most resistant microorganisms were molds and yeasts, followed by bacteria spores, sulfate-reducing clostridia and vegetative forms of bacteria.

The content of amino acid in gluten sterilized with various doses did not decrease.

The content of protein in gluten decreased as the dosage of ionic radiation increased.

The contents of both xanthophyll and carotin decreased with the increase of radiation dose except that treated by 10 kGy.

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