UDC 664.684 : 633. 888] : 579. 67

Original scientific paper

MICROBIOLOGICAL SAFETY OF STRUDEL FILLED WITH POPPY SEEDS AND PACKAGED IN MODIFIED ATMOSPHERE

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ABSTRACT: Variety of bakery products and their complex composition represents a special problem when it comes to their sustainability. Achieved product quality is necessary to preserve. One way to preserve the quality of bakery products which usually have high a_w value, is packaging into appropriate package. In terms of food production, there are different packaging materials and different methods of packaging. In this study, microbiological safety of basic and additional raw materials used in strudel making as well as packaging materials and finished product, strudel filled with poppy seeds were examined. The strudel was packaged in containers made of polypropylene and multi-layer coextruded film in atmospheric environment (ATM) and modified atmosphere (MAP). The packaged strudel filled with poppy seeds was stored at room temperature (23-25 C). The tests were performed on the day of production and successively after every 7 days following the dynamics 0,7,14,21,28,35 days.

Microbiological tests showed that all the basic and additional materials and packaging materials were microbiologically correct. Common characteristic for all packaged products, regardless of applied packaging atmosphere and packaging material, was the absence of pathogen microorganisms. Increase in the initial number of microorganisms during storage, as well as difference in the total number of microorganisms after 5 weeks of storage between the samples packaged with different packaging techniques was observed. The strudel packaged under MAP conditions in multi-layer coextruded film had a minimum number of microorganisms, aerobic sporogenic bacteria, yeasts and moulds after five weeks of storage.

Keywords*: microbiological safety, strudel with poppy seeds, a_w, MAP packaging*

Received: 7 April 2011

Accepted: 24 June 2011

INTRODUCTION

Bakery products are very sensitive substrate of organic origin in which physical and chemical processes occur during storage which for a consequence has a change of quality, organoleptic, chemical and nutritional value. Product diversity and complex composition represents a special problem when it comes to their protection. In addition, it is important to produce them in an adequate manner, it is essential to preserve product quality. To achieve this it is necessary to put products into suitable container that will provide protection against mechanical damage and from chemical and biochemical changes caused by external factors (Vlahovic, 1999). Big problem of bakery industry is the shelf-life

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of bakery products. Regardless of different types of additives that are added to products to extend their shelf-life, bakery products' shelf-life is short. Spoilage of these products is influenced by many factors, some of them are: high water content (high a_w), very high nutritional value, abundance of minerals, vitamins, proteins, fats and carbohydrates which actually make them an ideal nutrient medium for all kinds of microorganisms.

Strudel filled with poppy seeds is produced according to traditional recipes from raw materials obtained in domestic production by a special technological process. The raw materials used in technological process of production are classified as basic and additional materials. Basic raw materials are wheat flour, water, fresh baker's yeast and salt. Other ingredients include: poppy seeds, sugar, vegetable fat, fresh eggs, vanilla sugar and lemon peel.

To achieve microbiological proper and sustainable products, it is essential that all materials are microbiologically correct.

To obtain the original strudel from Vojvodina, high quality wheat flour T-500 should be used in production, which should, in addition to the parameters of quality, satisfy microbiological parameters as well. Due to its chemical composition, flour is a very convenient base for the development of different populations of microorganisms. The usual micro flora in flour is comprised of bacteria, yeasts and moulds (Stojanovic, Psodorov, 2007). The majority of microorganisms in the flour come from the wheat itself. Wheat grains are suitable environment for microbial growth. Therefore, the grain surface treatment is a necessary condition to obtain products of proper hygiene, especially flour, irrespective of the proportion of peripheral parts of the grain (Plavsic et al., 2007).

Water used in the production of strudel with poppy seeds should fulfil certain quality requirements, which means that it is microbiologicaly and chemically proper, without foreign tastes and odours, colourless and transparent, in other words it should fulfil all the conditions required by the Regulations on the hygienic quality of drinking water (Pravilnik o higijenskoj ispravnosti vode za piće "Sl. list SRJ", br. 42/1998 i 44/1999). Fresh baker's yeast (*Saccharomyces cerevisiae*), salt and other additional raw materials listed above, should primarily meet the requirements of microbiological safety, according to the Regulation on microbiological safety of food in trade (Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu, "Sl.list SRJ", br. 26/1993,53/1995 i 46/2002).

According to the traditional recipe for the production of strudel with poppy seeds, poppy seeds are first milled and added to the strudel in form of filling in the quantity of at least 60% dough basis. In nutritional purposes poppy seeds (*Papaver somniferum* L.) are used as raw material for production of edible oils and for making various types of cakes, usually with grinded poppy. Poppy comes from Asia Minor. From there it spread to the Far East and Europe. Poppy seeds can be red, gray, yellow, blue or black. The chemical composition of poppy seed is very rich: 5.8% water, 22% crude proteins, 47.5% oils, 10.2% carbohydrates, 7.8% crude fibres and about 6.2% mineral materials. Oils make the highest percentage, and content may vary depending on the subspecies from 39.67% to 48.92% (Sabados et al., 2010), while the percentage of oils in poppy seeds originating from Macedonia is between 52% and 61%. Preservation of poppy seeds is very important. When stored, it should not contain more than 10% water, or poppy seeds will quickly turn mouldy and rancid. Fine granulation makes a great contact surface for microorganisms. By grinding the seeds, the surface area is additionally increased. Because of their small size, poppy seeds, like spices, are subject to microbiological contamination, in terms of increased numbers of bacteria and fungi. Therefore, poppy seeds can be a source of microbiological spoilage of products in which they are included (Banerjee & Sarkar, 2003). Due to the large percentage of oil, poppy seeds are susceptible to oxidative changes, and as such may affect the safety of the final product in which they were used.

Oxidative processes are often the cause of changes in composition and organoleptic characteristics of packaged foods, especially baked goods. The effect of oxygen often causes undesirable changes in colour, taste and flavour, reduces the nutritional value of food and, in certain cases, can be toxic to humans (Vereš, 1991). Given the easy spoiling and great sensitivity of food products, containers intended for food packaging act like barriers. Selected packaging materials must be acceptable for human health and must meet the conditions foreseen by the Law on Health

Safety of Food and Items of General Use (Vujković, 1992; Vujković, 1983).

In terms of food production different packaging operations are used. They are dependent on: the type of food product, selected packaging and subsequent operations with packed content. The quality of the selected package is an important prerequisite for ensuring the shelf-life of packaged products.

To maintain product quality in the desired period, the containers must have defined quality characteristics and proper packaging operations must be applied.

For packaging sensitive bakery products packaging materials must be impermeable or slightly permeable to oxygen and water vapour, suitable for packing in protective atmosphere or vacuum. Since not all polymers have good barrier properties, multi-layer and combined materials manufactured with most advanced methods of laminating, extrusion coating and co-extrusion are used for food packaging (Lazic et al., 2000), (Lazic et al., 2008).

A procedure for packaging in controlled atmosphere(CAP), i.e. modified atmosphere (MAP) has been developed more recently (Smith et al., 1996). Packaging in protective atmosphere is a technique during which the proportion of oxygen in the area around the food is changed, usually decreased, and an inert gas or mixture of gases is flushed instead. In practice, firstly, a partial vacuuming of space around the product is performed (special attention is paid to the consistency of the product - the appearance and structure of the product must not be impaired), then inert gas is injected. In that way the oxygen content around the product is decreased. The choice of gas is dependant on the micro flora present in or on the product, the product's sensitivity to O_2 and CO_2, the stability of colour, odour and taste (Philips, 1996).

Oxygen can affect food in different ways – it can favour the growth of aerobic microorganisms and inhibit the growth of anaerobic microorganisms. Higher concentrations of O_2 can cause spoilage, especially in food with high content of fat, vitamin degradation and undesirable colour changes. Oxygen is responsible for the oxidation of food constituents and development and reproduction of microorganisms and insects. Modified atmosphere packaging and vacuum packaging are widely accepted in order to exclude oxygen from the free space in packagings. However, these physical methods of packaging usually do not s remove all the oxygen (Psodorov, 1999).

Nitrogen is used for packaging products with a large proportion of fat (strudel filled with poppy seeds contain about 8% fat) and exclusion of oxygen in order to prevent undesirable oxidative changes, to inhibit the growth of aerobic organisms and as a filling gas for packaging in high concentrations of CO_2, when absorption of CO_2 in the content over time occurs. Nitrogen behaves as an inert gas and has no microbiotic activity (Philips, 1996).

Carbon dioxide is the major microbiotic factor in the packaging technique by using protective atmosphere. It inhibits the growth of moulds and bacteria that cause spoilage. In order to have any effect, CO_2 must be added in relatively high concentrations (20% or more). Carbon dioxide reacts with water in the product and creates carbonic acid that lowers the pH value which inhibits the development and proliferation of most types of microorganisms. Influence of CO_2 is not universal and depends on the type of micro flora present in a food product (Kotsianis et al., 2002).

Because of the effects of gases like nitrogen and carbon dioxide, it is necessary to select a packaging material that will enable the preventive action of gases in the package against the development of micro flora, while, at the same time, they should not react with the content or external environment.

The selected packaging material must have adequate permeability, good appearance, good mechanical properties and a reasonable price. It must be suitable for packaging machines that use MAP technique.

MATERIAL AND METHODS

Microbiological safety of basic and additional raw materials used in the production of strudel with poppy seed filling was examined in the first phase of this study. Then, selection of packaging materials was performed.

Figure 1. Strudel with poppy seeds

Packaging was performed on a semi-automatic packaging machine (Multivac, Germany). Modified atmosphere was made by an automatic mixer of gases from mixture of CO_2 and N_2 in proportion 20:80. Manufacturing and packaging of strudel filled with poppy seeds was carried out in industrial conditions in "Kikinda AD" Bakery according to traditional recipe.

The packaged strudels with poppy seed filling were stored under ambiental conditions (23 °C to 25 °C). The tests were performed on the day of production and successively after every 7 days following the dynamics 0, 7, 14, 21, 28, 35 days.

The samples were tested in accordance to the Regulation on the Microbiological Safety of Food in Trade (Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu,"Sl.list SRJ", br. 26/1993,53/1995 i 46/2002)., Regulation on the Hygiene of Drinking Water (Pravilnik o higijenskoj ispravnosti vode za piće "Sl. list SRJ", br. 42/1998 i 44/1999) and Law on health safety of food and items of general use (Zakon o zdravstvenoj ispravnosti životnih namirnica i predmeta opšte upotrebe "Sl. list SFRJ", br. 53/91 i "Sl. list SRJ", br. 24/94, 28/96 i 37/2002 i "Sl. glasnik RS", br. 101/2005 - dr. zakon i 79/2005 - dr. zakon).

Determination of microbiological parameters was done according to the Methods of Carrying Out Microbiological Analysis and Super-Analysis (Pravilnik o metodama vršenja mikrobioloških analiza i superanaliza, "Sl. list SFRJ", br. 25/1980), according to official methods (method 1, method 3, method 4, method 7, method 8, method 9, method 10, method 11, method 12) and according to the Regulation on sampling method and methods for laboratory analysis of drinking water (Pravilnik o načinu uzimanja i metodama za laboratorijsku analizu vode za piće, "Sl. list SFRJ", br. 33/1987-Prilog III), (method 1, method 3, method 4, method 5, method 6, method 7).

RESULTS AND DISCUSSION

In order to achieve microbiologically safe and sustainable product, it is essential that all raw materials are microbiologically correct.

Tables 1 and 2 show the results of microbiological safety of basic and additional raw materials.

According to the Regulation on microbiological safety of food in trade (Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu, "Sl. list SRJ", br. 26/1993, 53/1995 i 46/2002), the products are microbiologically valid if the sample does not contain the following pathogenic microorganisms: coagulase positive *Staphylococcus*, sulphitereducing clostridia, *Proteus* sp. and *Escherichia coli*.

Based on the results shown in Tables 1 and 2 above, no pathogenic microorganisms were isolated. The count of microorganisms and yeasts and moulds was in the acceptable limits according to the Regulation (Pravilnik o mikrobiološkoj ispravnosti namirnica u prometu,"Sl. list SRJ", br. 26/1993, 53/1995 i 46/2002). Lipolytic microorganisms were not isolated.

Table 1.
Microbiological safety of basic raw materials

Microbiological parameters	Basic raw materials			
	Wheat flour T-500	Water	Fresh baker's yeast	Salt
Salmonellae sp.	ND[a]		ND[a]	ND[a]
Coagulase positive *Staphylococcus*	ND[a]		ND[a]	ND[a]
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	ND[a]
Proteus sp.	ND[a]	ND[a]	ND[a]	ND[a]
Escherichia coli	ND[a]	ND[a]	ND[a]	ND[a]
Total count of moulds and yeast in 1g	50			ND[a]
Total count of microorganisms in 1g		ND[a]		ND[a]
Coliform bacteria		ND[a]		
Pseudomonas aeruginosa		ND[a]		
Streptococcus faecalis		ND[a]		

Legend: ND[a] - not detected

Table 2.
Microbiological safety of additional raw materials

Microbiological parameters	Additional raw materials						
	Poppy seeds	White granulated sugar	Milk	Vegetable fat	Fresh eggs	Vanilla sugar	Lemon peel
Salmonellae sp.	ND[a]	ND[a]	ND[a] -	ND[a]	ND[a]	ND[a]	ND[a]
Coagulase positive *Staphylococcus*	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Proteus sp.	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Escherichia coli	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Total count of moulds and yeast in 1g	ND[a]	ND[a] -			ND[a]	ND[a]	
Total count of microorganisms in 1g	$1x10^3$	ND[a]	$1x10^2$		ND[a]	ND[a]	
Lypolytic microorganisms	ND[a]			ND[a]			

Legend: ND[a] - not detected

Table 3.
Microbiological safety of packaging materials

Microbiological parameters	Packaging materials	
	Polypropylene film PP	Multilayer co-extruded film C
Salmonellae sp.	ND [a]	ND [a]
Coagulase positive *Staphylococcus*	ND [a]	ND [a]
Sulphite reducing clostridia	ND [a]	ND [a]
Proteus sp.	ND [a]	ND [a]
Escherichia coli	ND [a]	ND [a]
Total count of microorganisms in 1g	ND [a]	ND [a]

Legend: ND [a] - not detected

Table 4.
Microbiological safety of strudel with poppy seeds packaged in polypropylene film under atmospheric conditions

Microbiological parameters	Storage period				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Salmonellae sp.	ND[a]	ND[a]	ND[a]	/	/
Coagulase positive *Staphylococcus*	ND[a]	ND[a]	ND[a]	/	/
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	/	/
Proteus sp.	ND[a]	ND[a]	ND[a]	/	/
Escherichia coli	ND[a]	ND[a]	ND[a]	/	/
Total count of moulds and yeast in 1g	ND[a]	$5,0x10^2$	$2,0x10^3$	/	/
Total count of aerobic sporogenic bacteria in 1g	$1,0x10^2$	$2,0x10^3$	$5,0x10^3$	/	/
Total count of microorganisms in 1g	$1,0x10^3$	$5,0x10^4$	$2,0x10^5$	/	/

Legend: ND[a] - not detected

Table 5.
Microbiological safety of strudel with poppy seeds packaged in multi-layer coextruded film under atmospheric conditions

Microbiological parameters	Storage period				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Salmonellae sp.	ND[a]	ND[a]	ND[a]	/	/
Coagulase positive *Staphylococcus*	ND[a]	ND[a]	ND[a]	/	/
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	/	/
Proteus sp.	ND[a]	ND[a]	ND[a]	/	/
Escherichia coli	ND[a]	ND[a]	ND[a]	/	/
Total count of moulds and yeast in 1g	10	$2,0x10^2$	$1,0x10^3$	/	/
Total count of aerobic sporogenic bacteria in 1g	20	$1,0x10^3$	$2,0x10^3$	/	/
Total count of microorganisms in 1g	$1,5x10^3$	$3,0x10^4$	$95x10^3$	/	/

Legend: ND[a] - was not detected

Table 6.
Microbiological safety of strudel with poppy seeds – packaged in polypropylene film under modified atmosphere

Microbiological parameters	Storage period				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Salmonellae sp.	ND[a]	ND[a]	ND[a]	/	/
Coagulase positive *Staphylococcus*	ND[a]	ND[a]	ND[a]	/	/
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	/	/
Proteus sp.	ND[a]	ND[a]	ND[a]	/	/
Escherichia coli	ND[a]	ND[a]	ND[a]	/	/
Total count of moulds and yeast in 1g	ND[a]	$4,0x10^2$	$3,0x10^3$	/	/
Total count of aerobic sporogenic bacteria in 1g	50	$5,0x10^2$	$1,5x10^3$	/	/
Total count of microorganisms in 1g	150	$1,5x10^3$	$16x10^3$	/	/

Legend: ND[a] - not detected

Table 7.
Microbiological safety of strudel with poppy seeds – packaged in multi-layer coextruded film under modified atmosphere

Microbiological parameters	Storage period				
	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Salmonellae sp.	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Coagulase positive *Staphylococcus*	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Sulphite reducing clostridia	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Proteus sp.	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Escherichia coli	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Total count of moulds and yeast in 1g	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Total count of aerobic sporogenic bacteria in 1g	10	20	ND[a]	10	10
Total count of microorganisms in 1g	$1,0x10^2$	$3,0x10x^2$	$1,0x10^2$	$2,0x10^2$	$3,0x10x^2$

Legend: ND[a] - not detected

Water used in the food industry needs to match the quality of drinking water. Based on the results in Table 1, water meets the microbiological Regulation on hygienic quality of drinking water (Pravilnik o higijenskoj ispravnosti vode za piće "Sl. list SRJ", br. 42/1998 i 44/1999).

Table 3 presents the results of microbiological safety of packaging materials.

Microbiological safety of packaging materials was tested by method of swabbing. Packaging used in the food industry is considered an item of general use, and according to the Law on health safety of food and items of general use should not contain pathogenic microorganisms harmful to human health (Zakon o zdravstvenoj ispravnosti životnih namirnica i predmeta opšte upotrebe "Sl. list SFRJ", br. 53/91 i "Sl. list SRJ", br. 24/94, 28/96 i 37/2002 i "Sl. glasnik RS", br. 101/2005 - dr. zakon i 79/2005 - dr. zakon).

The results presented in Table 3 show that the packaging materials are microbiologically safe. Tables 4 and 5 show the results of microbiological safety of strudel with poppy seeds packaged in containers from polypropylene film and multi-layer coextruded film packa-ged under atmospheric conditions (ATM).

Tables 6 and 7 show the results of microbiological safety of strudel filled with poppy seeds packaged in containers made of polypropylene film and multi-layer coextruded film packed in modified atmosphere (MAP).

A common characteristic of all packaged products, regardless of packaging atmosphere and the applied packaging technique, is that strudel was not contaminated with pathogenic microorganisms. However, it is obvious that there is some difference in the total number of microorganisms between the samples packaged with different packaging techniques; the total count of microorganisms is minimal in the MAP packaged samples. This can be interpreted by bactericidal effect of CO_2 in the modified atmosphere. Carbon dioxide reacted sourly, lowering pH value on product surface which prevented the growth and proliferation of yeasts, moulds and bacteria. Owing to the MAP technique, properly selected atmosphere composition and packaging material, it is apparent from Tables 6 and 7 that the MAP packaged products contain minimum total count of microorganisms which led to shelf-life extension for more than a month.

Shelf-life extension and prevention of spoilage of strudels are two main objectives in the MAP packing atmosphere. It is very important not to allow the pathogenic conditions that will cause spoiling of strudel (in recipes where eggs and milk are used); they may also occur due to the occurrence of toxins or secondary infection of strudel. It is therefore important to suppress the growth of pathogenic species and inactivate those that can be found in the strudel. When considering the shelf-life extension, the total destruction and growth inhibition of microorganisms is not always necessary. When microbial growth is not a limiting factor for shelf-life, immobilizing enzymes may play a role in the lifetime extension, by causing the limiting reaction, such as oxidation, by removing catalysts or reactants.

CONCLUSIONS

Based on testing of basic and additional raw materials, formed packaging and packed strudel with poppy seeds, and the obtained and discussed results, the following can be concluded:

- Microbiological tests showed that all the basic and additional raw materials were microbiologically safe.
- Microbiological examination revealed that the formed containers and packaging materials were microbiologically safe.
- Microbiological examination revealed the initial growth of microorganisms, with obvious difference in the total number of microorganisms after 5 weeks of storage.
- The samples packaged in MAP contained minimum number of microorganisms, aerobic spore bacteria, yeasts and moulds after 5 weeks of storage.
- The samples packaged in modified atmosphere were preserved for a longer period (35 days), as a result of excellent barrier properties of applied packaging, primarily because of low permeability to gases.
- Based on the results of testing, for packaging strudel filled with poppy seeds in industrial conditions with shelf-life up to 30 days on room temperature, it is recommended to use modified atmosphere packaging in multi-layer co-extruded film sample 2 - MAP.

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МИКРОБИОЛОШКА ИСПРАВНОСТ ШТРУДЛЕ СА МАКОМ ПАКОВАНЕ У ИЗМЕЊЕНОЈ АТМОСФЕРИ

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Разноврсност пекарских производа и њихов сложени састав представљају посебан проблем када је у питању њихова одрживост. Постигнути квалитет производа потребно је и сачувати. Један од начина очувања квалитета пекарских производа који по правилу садрже високу a_w вредност је паковање у одговарајућу амбалажу. У условима производње хране користе се различити амбалажни материјали и примењују различити поступци паковања. У овоме раду испитана је микробиолошка исправност основних и додатних сировина које се користе у изради штрудле са маком, као и амбалажни материјал и готов производ, штрудла са маком. Произведена штрудла пакована је у амбалажу од полипропиленске и вишеслојне коекструдиране фолије у атмосферским условима (АТМ) и модификованој атмосфери (МАП). Упакована штрудла са маком складиштена је у собним условима (23-25 C). Испитивања су вршена на дан производње и сукцесивно након сваких 7 дана по динамици 0, 7, 14, 21, 28, 35 дана.

Микробиолошким испитивањем утврђено је да су све основне и додатне сировине, као и амбалажни материјали били микробиолошки исправни. Заједничка карактеристика свих упакованих производа, без обзира на атмосферу паковања и на примењену амбалажу, јесте та да узорци штрудле са маком нису били контаминирани патогеним микроорганизмима. Утврђен је пораст иницијалног броја микроорганизама, као и очигледна разлика у укупном броју микроорганизама након 5. недеље скадиштења. Штрудла упакована у условима МАП атмосфере у амбалажу од вишеслојне коекструдиране фолије, имала је минималан број микроорганизама, аеробних спорогених бактерија, квасац и плесни после пет недеља складиштења.