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RESEARCH NOTE

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Physical and Microbiological Changes of Sliced Process Cheese Packaged in Edible Pouches during Storage

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Abstract The objectives of this study were to compare the quality changes of cheese slices individually packed in four kinds of edible pouches in order to select the most suitable variety for individual packaging. The edible Z2 pouch (zein with oleic acid) was selected as maintaining the best cheese qualities based on the physical and microbiological changes undergone by the samples over 4-week storage at 5°C. The cheese sample individually packed in Z2 inner edible pouch and repacked in a plastic (OPP/LLDPE) outer pouch was not significantly different in physical and microbiological changes from that individually packed in a plastic (OPP/LLDPE) inner pouch and repacked in a plastic (OPP/LLDPE) outer pouch. Therefore, it may be concluded on the basis of the physical and microbiological evidence that the Z2 edible pouch can be used as an inner package for cheese slices when it is inside a plastic outer pouch.

Key words: edible pouches, plastic pouches, cheese slices, physical and microbiological changes

Introduction

Process cheese is usually produced by heat-assisted grinding and blending various cheese types into a uniform and pliable mass, with the advantages of compact body, smooth texture, improved slicing properties, smooth melting, possibility of controlling sensory and physical properties, and varieties of shapes and forms (1). Despite these advantages, however, various quality changes can occur during its storage including microbiological, physical (including textural), and chemical (including nutritional) changes. One of the most important quality factors is the moisture content or water activity of process cheese, which affects the physical (including textural) and microbiological qualities (2).

In order to minimize the undesirable quality changes, commercial sliced process cheese is individually packed in a barrier laminate (OPP/LLDPE, nylon/LLDPE, PET/PE, etc.) and further re-packed in another barrier laminate (OPP/LLDPE, nylon/LLDPE, PET/PE, etc.) under modified atmosphere (2). This individual packing process requires a lot of plastic packaging material which later become a non-degradable plastic waste. For the individually packaging of one slice (8 cm × 8 cm and 20 g each), 0.0128 m^2 of plastic packaging material is consumed; while, for one metric ton of slices, 640 m^2 of plastic film is needed. Such a large amount of non-degradable plastic film can be effectively replaced by edible films composed of biological macromolecules such as polysaccharide, protein, and lipid for the sake of environment. Such an edible film must satisfy certain requirements in terms of barrier property, physical strength, and heat-sealability.

High amylose corn starch (HACS) is an interesting edible polysaccharide, which can produce films with higher barrier properties and physical strength than normal corn starch films (3). Zein is also an important edible protein, able to produce films with heat-sealability and barrier properties (4). The moisture barrier function of both single HACS and zein films degrades with the increased use of plasticizers (7). Therefore, it is desirable to add lipids such as fatty acids to HACS and/or zein to improve the barrier properties of individual film-forming material (5). Recently, Ryu *et al.* (7) prepared edible films made from HACS, zein and/or fatty acid, and determined their physical properties. In the present study, these edible films were made into pouches and applied for the individual packing of processed cheese slices.

The objectives of this study were as follows. Firstly, to individually pack the sliced process cheese using four kinds of edible pouches made from HACS and/or zein with or without lipid. Secondly, to compare the physical and microbiological quality changes of the cheese slices individually packed in edible pouches with those packed in plastic pouches during 4-week storage at 5°C and to select the most appropriate edible pouch. Thirdly, to repack the sliced process cheese individually packed in the selected edible pouch with a plastic outer package. Fourthly, to compare the physical and microbiological quality changes of the cheese slices individually packed in the selected edible pouch and repacked in a plastic pouch with those individually packed in a plastic pouch and repacked in another plastic pouch during 4-week storage at 5°C.

Materials and Methods

Materials HACS (HYLON VII, amylose content 70%) was obtained from National Starch & Chemical Co. (Bridgewater, NJ, USA) and zein from Showa Sangyo Inc. (Tokyo, Japan). Sorbitol was purchased from Cerestar NV/SA (Paris, France), 95% alcohol and oleic acid from

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DukSan Chemicals, and polyethylene glycol (PEG) 400 from Sigma Chemicals. Process cheese samples and commercial plastic cheese packaging materials (OPP/LLDPE, 30 μm for inner package and 60 μm for outer package) were obtained from Namyang Dairy Products Co., Ltd. (Chungnam, Korea).

Edible pouch preparation Four kinds of edible films were made according to the procedure described by Ryu *et al.* (7). The films were Z1 (zein film with PEG and glycerol), Z2 (zein film with oleic acid), ZA1 (zein-coated HACS film with PEG and glycerol), and ZA2 (zein-coated HACS film with oleic acid). When making Z1 and Z2 films, either zein (16.7 g) and glycerol : PEG 400 (1:1) (3.34 g), or zein (16.7 g) and oleic acid (3.34 g), respectively, were weighed in 100 g 95% ethanol and dissolved by heating at 85°C for 20 min. The mixture was cast on the polystyrene plate (120 mm × 120 mm, 10 mm depth), and then dried at 50°C. Before making ZA1 and ZA2 films, a suspension of 3 g HACS with 0.6 g sorbitol in 100 g water was preheated at 100°C for 30 min, gelatinized at 160°C in an oil bath for 30 min, cooled to 80°C, cast on the polypropylene plate (300 mm diameter and 10 mm depth), and dried at 50°C for 24 hours. Then the ZA1 and ZA2 films were prepared by dipping the above HACS films in 100 g alcoholic solution (85°C), either with zein (16.7 g) and glycerol : PEG 400 (1:1) (3.34 g), or zein (16.7 g) and oleic acid (3.34 g), respectively, and drying at 50°C for 4 hours.

The above films were made into pouches as follows. The three sides of the dried film were heat-sealed for 0.5 second in a packaging machine (Leepack M-1AG, The Korea Electronic MFG., Corp., Incheon, Korea), during which a parchment paper was placed on the surface of the heating bar to prevent the film from adhering to it.

Packing of cheese slices Some sliced cheese samples (8 cm × 8 cm × 3 mm and 20 g each) were removed from the inner and outer plastic packages (OPP/LLDPE, 30 μm and 60 μm, respectively), placed in the individual, edible pouches, Z1, Z2, ZA1, and ZA2, and then heat sealed using the same Leepack M-1AG packaging machine. Other sliced cheese samples in intact inner plastic pouches (OPP/LLDPE, 30 μm) were removed from the outer plastic packages and were used as a reference for comparing the physical and microbiological quality changes. The most appropriate edible pouch was selected based on the storage test results. Then some sliced cheese samples were removed from the inner and outer plastic packages, placed in the selected edible pouch, and heat-sealed using the Leepack M-1AG packaging machine. Next, the individually packed samples were placed again in plastic pouches (OPP/LLDPE, 60 μm), evacuated, and sealed in the Leepack M-1AG packaging machine with the injection of nitrogen gas provided from DD Gas Corporation (Incheon, Korea). Other sliced cheese samples in inner and outer plastic pouches (OPP/LLDPE, 30 μm and 60 μm, respectively) were used as a reference for comparing the physical and microbiological quality changes during storage.

Physical and microbiological analyses The four groups of packaged cheese samples were kept at 5°C for 4 weeks and taken out every week for physical and microbiological analyses. The moisture content of the cheese samples was determined by vacuum drying for 24 hours at 70°C and a pressure of 100 mmHg. The texture of the cheese samples was measured with a texture analyzer (TA-XT2, Stable Micro Systems, London, England) with 5 kg load cell, 1 mm compression and 0.1 mm/sec crosshead speed. Hardness was determined in triplicate from the texture profile curves. The cheese samples were examined on the Plate Count Agar for bacteria counts, Czapek Dox Solution Agar for mold counts, and YPD Agar for yeast counts. Each sample was diluted (10^{-1}) with sterilized water and incubated at 30°C for 36 hours. The colonies were counted according to standards for grades of dry milks (6).

Statistical analysis Statistical differences were analyzed using the Duncan's multi-range test of SAS (Statistical Analysis System).

Results and Discussion

Comparison of quality changes of sliced cheese individually packed in edible pouches with those in plastic pouches Table 1 lists the changes in moisture content, hardness, bacteria counts, mold counts, and yeast counts of the sliced processed cheese individually packed in edible pouches (Z1, Z2, ZA1, and ZA2) and in plastic pouches (OPP/LLDPE) and stored at 5°C for four weeks. The sliced cheese samples individually packed in edible pouches rapidly began to lose moisture from the beginning of the storage period. At day 7 of storage, the Z2 pouch sample showed the lowest moisture loss, followed by ZA2, ZA1, and Z1 pouch samples. In contrast, the sliced cheese samples individually packed in plastic pouches showed little changes in moisture content during storage. The difference in moisture loss was mainly due to the difference in the moisture barrier properties of edible and plastic films. According to Ryu *et al.* (7) the plastic film (OPP/LLDPE) had the lowest water vapor permeability (WVP), followed by Z2, Z1, ZA2, and ZA1. The rate of moisture loss corresponded to the WVP values, except for Z1. The exception may be attributed to the fact that the Z1 film had a loose and rough surface structure, as compared to the other films, which seemed responsible for the relatively high moisture loss through the Z1 film during 4-week storage (8). At day 14, the Z2 pouch sample still showed the lowest moisture loss; however, the other three pouch samples did not show any significant difference in the degree of moisture loss. At day 21 and 28 none of the edible pouch samples showed any significant difference in the rate of moisture loss.

The edible pouch samples rapidly became hardened from the beginning of storage. At day 7, the Z2 pouch sample showed the slowest hardening, followed by ZA2, ZA1, and Z1 pouch samples. In contrast, the plastic pouch sample showed little changes in hardness. In general the hardness increased as moisture content decreased; therefore, the difference in hardness seemed directly related to the difference in the moisture barrier properties of the edible and plastic films as mentioned earlier. From the second week, none of the edible pouch samples

Table 1. Changes in moisture content, hardness, bacteria counts, mold counts, and yeast counts of sliced processed cheese individually packed in four kinds of edible pouches vs. OPP/LLDPE plastic pouch and stored at 5°C for four weeks

Experimental items	Pouches[1)]	Storage time at 5°C (weeks)				
		0	1	2	3	4
Moisture content (%)	Edible pouch Z1	47.49±0.16	9.62±0.58^{e}	7.53±0.39^{e}	6.92±0.45^{e}	5.60±0.21^{e}
	Edible pouch Z2		29.04±0.50^{b}	12.23±0.25^{f}	7.89±0.39^{e}	6.88±0.22^{e}
	Edible pouch ZA1		12.80±0.51^{d}	7.71±0.36^{e}	7.20±0.28^{e}	6.16±0.23^{e}
	Edible pouch ZA2		15.88±0.49^{c}	8.05±0.59^{e}	7.43±0.18^{e}	6.31±0.25^{e}
	Plastic pouch		47.34±0.16^{a}	47.24±0.14^{a}	47.20±0.12^{a}	47.19±0.09^{a}
Hardness (N)	Edible pouch Z1	1.35±0.03	134.92±2.70^{e}	155.00±3.43^{e}	180.21±3.89^{e}	194.72±3.78^{e}
	Edible pouch Z2		66.74±2.18^{b}	142.52±3.41^{e}	162.46±3.74^{e}	184.81±3.80^{e}
	Edible pouch ZA1		121.68±2.43^{d}	148.07±3.19^{e}	172.28±3.81^{e}	187.83±3.79^{e}
	Edible pouch ZA2		98.00±2.53^{c}	143.09±2.80^{e}	167.38±3.88^{e}	185.82±3.68^{e}
	Plastic pouch		1.42±0.05^{a}	1.49±0.06^{a}	1.67±0.03^{a}	1.72±0.02^{a}
Bacteria counts (log CFU/g solid)	Edible pouch Z1	1.73±0.48	2.32±0.40^{a}	2.34±0.41^{a}	2.40±0.48^{a}	2.67±0.65^{a}
	Edible pouch Z2		2.28±0.34^{a}	2.38±0.45^{a}	2.38±0.48^{a}	2.68±0.70^{a}
	Edible pouch ZA1		2.20±0.28^{a}	2.26±0.30^{a}	2.30±0.34^{a}	2.62±0.72^{a}
	Edible pouch ZA2		2.15±0.26^{a}	2.30±0.34^{a}	2.34±0.46^{a}	2.63±0.72^{a}
	Plastic pouch		1.73±0.60^{a}	1.84±0.48^{a}	1.85±0.78^{a}	2.51±0.85^{a}
Mold counts (log CFU/g solid)	Edible pouch Z1	1.68±0.42	1.90±0.46^{a}	2.00±0.45^{a}	2.20±0.56^{a}	2.33±0.68^{a}
	Edible pouch Z2		1.88±0.44^{a}	1.98±0.58^{a}	2.19±0.68^{a}	2.32±0.50^{a}
	Edible pouch ZA1		1.85±0.48^{a}	1.96±0.38^{a}	2.18±0.54^{a}	2.31±0.63^{a}
	Edible pouch ZA2		1.81±0.29^{a}	1.94±0.49^{a}	2.16±0.57^{a}	2.30±0.63^{a}
	Plastic pouch		1.78±0.60^{a}	1.88±0.68^{a}	1.93±0.70^{a}	2.10±0.50^{a}
Yeast counts (log CFU/g solid)	Edible pouch Z1	0.85±0.48	2.15±0.49^{a}	2.52±0.39^{a}	2.53±0.67^{a}	2.56±0.53^{a}
	Edible pouch Z2		1.90±0.30^{a}	2.49±0.57^{a}	2.51±0.47^{a}	2.54±0.73^{a}
	Edible pouch ZA1		1.65±0.50^{a}	2.40±0.72^{a}	2.46±0.32^{a}	2.48±0.53^{a}
	Edible pouch ZA2		1.60±0.29^{a}	2.30±0.53^{a}	2.38±0.42^{a}	2.45±0.50^{a}
	Plastic pouch		1.11±0.60^{a}	1.77±1.04^{a}	2.08±0.78^{a}	2.30±1.51^{a}

showed any significant difference in the rate of hardening. The edible pouch samples showed no significant differences in bacteria counts during storage. The initial bacteria count (1.73 log CFU/g solid) increased to 2.24 log CFU/g solid at day 7, remained stable, and then increased again to 2.65 log CFU/g solid at day 21 and 28, with a net increase of 0.9 log range. In the case of the plastic pouch sample, the bacterial counts remained stable at 1.81 log CFU/ g solid for 3 weeks and then increased to 2.51 log CFU/ g solid sat day 28, with a net increase of 0.8 log range. All the edible pouch samples had bacteria counts higher than the plastic pouch sample during the 4 weeks of storage, although the differences were not significant. The edible pouches themselves seemed to be one of the sources of bacteria count increase, but the increase was not significant. According to Muir *et al.* (11), the total bacteria counts (1.0~3.4 log CFU/g) of processed cheese analogues were low and unlikely to have any practical consequences.

In the case of mold counts, the edible pouch samples showed no significant differences during storage. The initial mold count (1.73 log CFU/g solid) increased during storage to around 2.32 log CFU/g solid at day 28, with a net increase of 0.6 log range. In the case of the plastic pouch sample, the mold count increased to 2.10 log CFU/g solid at day 28, with a net increase of 0.4 log range. The edible pouch samples showed mold counts that were higher but not significantly different from the plastic pouch sample.

No significant differences in yeast count changes were noticed among the edible pouch samples. The initial yeast count of the edible pouch samples increased from 0.95 log CFU/g solid during storage to 2.54 log CFU/g solid at day 28, with a net increase of 1.6 log range. In the case of the plastic pouch sample, the yeast count increased to 2.26 log CFU/g solid at day 28, with a net increase of 1.3 log range. All the edible pouch samples showed higher yeast counts than the plastic pouch sample, but the differences were not significant.

The four edible pouch samples showed no significant differences in microbiological changes, although the Z2 pouch sample showed the lowest moisture loss over the first two weeks and the slowest hardening over the first week. The Z2 pouch was therefore selected as the most suitable edible pouch for the inner packaging of sliced cheese.

Quality changes of sliced cheese individually packed in an edible pouch and repacked in a plastic outer pouch Table 2 lists the changes in moisture content, hardness, bacteria counts, mold counts, and yeast counts of processed cheese slices individually packed in either edible pouch Z2 or plastic (OPP/LLDPE) inner pouch, then repacked in a plastic (OPP/LLDPE) outer pouch, and stored at 5°C for four weeks.

The slice with the Z2 edible inner pouch showed no significant difference in moisture loss from the sample individually packed in the plastic (OPP/LLDPE) inner pouch. Both cheese samples exhibited a moisture loss of 0.24~0.30% during storage, which was within the reported typical moisture loss range of 0.2~0.5% at 4°C (9).

The sample individually packed in the Z2 edible inner pouch was not significantly different in hardness changes

Table 2. Changes in moisture content, hardness, bacteria counts, mold counts, and yeast counts of sliced processed cheese individually packed in an edible Z2 inner pouch vs. OPP/LLDPE plastic pouch, re-packed in OPP/LLDPE plastic outer pouch and stored at 5°C for four weeks

Experimental items	Pouches[1)]	Storage time at 5°C (weeks)				
		0	1	2	3	4
Moisture content (%)	Edible pouch Z2 in Plastic pouch	47.49±0.16	47.36±0.17[a]	47.25±0.15[a]	47.21±0.13[a]	47.21±0.11[a]
	Plastic pouch in Plastic pouch		47.41±0.15[a]	47.31±0.14[a]	47.26±0.11[a]	47.25±0.08[a]
Hardness (N)	Edible pouch Z2 in Plastic pouch	1.35±0.03	1.39±0.04[a]	1.47±0.04[ab]	1.64±0.03[b]	1.69±0.02[b]
	Plastic pouch in Plastic pouch		1.38±0.06[a]	1.45±0.08[ab]	1.62±0.03[b]	1.68±0.03[b]
Bacteria counts (log CFU/g solid)	Edible pouch Z2 in Plastic pouch	1.73±0.48	2.27±0.36[a]	2.35±0.46[a]	2.36±0.52[a]	2.67±0.72[a]
	Plastic pouch in Plastic pouch		1.73±0.68[a]	1.79±0.44[a]	1.81±0.68[a]	2.48±0.80[a]
Mold counts (log CFU/g solid)	Edible pouch Z2 in Plastic pouch	1.68±0.42	1.83±0.0.44[a]	1.96±0.57[a]	2.18±0.67[a]	2.31±0.50[a]
	Plastic pouch in Plastic pouch		1.78±0.58[a]	1.86±0.63[a]	1.90±0.53[a]	2.08±0.42[a]
Yeast counts (log CFU/g solid)	Edible pouch Z2 in Plastic pouch	0.85±0.48	1.88±0.26[a]	2.48±0.36[a]	2.49±0.49[a]	2.53±0.43[a]
	Plastic pouch in Plastic pouch		1.00±0.62[a]	1.60±0.58[a]	1.99±0.61[a]	2.26±0.88[a]

from the sample individually packed in the plastic inner pouch. Both cheese samples showed a hardness increase from 1.4 N to 1.7 N, which was within the hardness value (2.0 N) of process cheese reported by Chen *et al.* (10).

The sliced cheese sample individually packed in the Z2 edible inner pouch had bacteria, mold, and yeast counts higher than the sample individually packed in the plastic inner pouch during storage. However, the net increases were in the log ranges of 0.9 for bacteria, 0.6 for mold, and 1.7 for yeast, and these differences were not significant.

Therefore, it may be concluded on the basis of the physical and microbiological results that the Z2 edible pouch can be used as an inner package for cheese slices when it is inside a plastic outer pouch.

Conclusion

In the absence of a plastic outer pouch, the cheese slices individually packed only in edible pouches showed relatively low moisture content and high hardness values in comparison to those individually packed in OPP/LLDPE plastic pouches. Among the four edible pouches tested, the Z2 pouch was the most suitable as the inner packaging material due to its slowest rate of moisture loss and hardening. The cheese slices individually packed in Z2 edible inner pouches and repacked in plastic outer pouches were not significantly different in moisture content, hardness, and microbiological counts from the samples individually packed in plastic inner pouches and repacked in plastic outer pouches. Therefore, it may be concluded on the basis of the physical and microbiological findings that the Z2 edible pouch can be used as an inner package of cheese slices when it is inside a plastic outer pouch.

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