

## CHANGES OF PECTIN IN APPLE MASS DEPENDING ON STORAGE TIME

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### Abstract

*Determination of pectin quantity enables to reduce volume of added binding matters, which provides not only the usage of all natural pectin found in plants, but also reduces content of chemically formed gradients in the product with this reducing its volume in the consumers' body. Pectin is a traditional agent for jams and jellies.*

*The aim of the research is to compare changes of pectin quantity in fresh, heat- treated and frozen apple products depending on storage time.*

*The apple varieties "Baltais Dzidrais", "Antonovka", "Zarja Alatau" harvested in Latvia State Institute of Fruit Growing were used for the experiment. Pectin, pH and soluble dry matter were determined in fresh, heat-treated and frozen apple mass for 3 months period of storage time. The results indicate that pectin quantity in apple mass is changing depending on the apple variety, way of preparation and storage time.*

**Key words:** apple mass, ripening, pectin, freezing, storage

### 1. INTRODUCTION

Fruits are considered as a commercially important and nutritionally essential food commodity due to providing not only the major dietary source of vitamins, sugars, organic acids, and minerals, but also other phytochemicals including dietary fiber and antioxidants with health-beneficial effects. Importance of fruit and vegetable consumption in a healthy nutrition is emphasized in the Commission's White Paper in relation to nutrition and health issues. The youth's nutrition has changed essentially and fruit consumption is considerably lower than it used to be before.

Apples are fruits that contain the highest pectin quantity. They are easily available and found in different varieties of apples along with being highly resourceful for daily necessities. Pectin, according to its significance in diet, is a dietary fiber, the entity "non-starch polysaccharide", which practically is not decomposed and assimilated in human's digestive tract. Pectin is food fiber, which clears the digestive tract from heavy metals and final products of metabolism - low molecular organic compounds. Pectin substances are used as food additives – thickeners, gelling agents and stabilizers (Kreicbergs V., 2008). These polysaccharides make new specific products on fruit basis and improve existing traditional products according to customer needs. The food additives are used to carry out certain functions of food products. At present one of the branches of food production, where there are many unused applications of food hydrocolloids, is fruit processing, which by all means occupies a remarkable part of food industry in Latvia.

On the basis of worked out functional analysis of colloids, it is possible to optimize regulation possibilities of heat processes for different ripeness stages of apples. It is established that ripeness stage influences pectin quantity in fruits, but there are no data about pectin quantity changes in frozen

fruits. It is important to measure pectin in fresh, heat- treated and frozen apple mass, in order to determine its potential jellying ability.

The aim of the research is to compare changes of pectin quantity in fresh, heat- treated and frozen apple products depending on storage time. The research is related to application possibilities of the current colloids, which are used in food industry at present.

## 2.MATERIAL AND METHODS

### 1. *Materials*

The apple varieties - 'Baltais Dzidrais', 'Antonovka' and 'Zarja Alatau' harvested in Latvia State Institute of Fruit Growing were used for the experiment. The experiment was carried out during the years 2010 - 2011.

The variety 'Baltais Dzidrais' is one of the best early summer varieties, which ripens in the beginning or mid-August. The fruit flesh is white, juicy, and soft, of pleasant sweet-and-sour flavour. Storage period of fresh apples is only one month. The varieties 'Antonovka' and 'Zarja Alatau' are late autumn-winter apple varieties, whose popularity can be explained by their resistance to cold winters and excellent storage feasibilities. Apples of the variety 'Antonovka' are big, green and upon ripening become yellow. Apples of the variety 'Zarja Alatau' are of variable size, beautiful yellowish colour, firm flesh. 'Antonovka' can be stored at least for 3 months but the storage time of 'Zarja Alatau' is 6 months.

#### 1.1. *Sample preparation*

Before carrying out the experiment apples were stored in an apple cellar, where the air temperature was +3° to +4°C and air humidity 80%. Thus apples maintained their characteristic properties.

In order to obtain indices of apples, they were blended unpeeled and grated through a sieve for obtaining a homogenous mass. Heat- treated and frozen apple mass was obtained by cutting apples in pieces, heating them up to the temperature +70°C and grating through a sieve. The grated apple mass for freezing was put into 200 ml big plastic ware and quickly cooled. Apple mass was stored at -18°C. Before carrying out analyses, frozen apple mass was thawed for an hour up to the room temperature.

The frozen apple mass was analyzed every month of the three month period. The fresh and heated apple mass from the varieties 'Antonovka' and 'Zarja Alatau' also were analyzed every month of the three month period. But apple mass from the variety 'Baltais Dzidrais' was analyzed every week during a month.

#### 1.2. *Method*

Pectin, pH and soluble dry matter were determined in fresh, heated (+70°C) and frozen apple mass.

Photometric measurement was used to determine the composition of pectin. Pectin was isolated from the apple mass by leaching with ethanol, and from the residues - by extracting with diluted sodium hydroxide solution. By adding carbasol and sulfuric acid to the extract, through different intermediate stages caroty condensation product was formed, which was photometrically measured at 525 nm. (Matiseks et.al, 1998)

For soluble dry matter measurement hand digital refractometer ATAGO N20 was used (ISO 2173:2003).

PH was determined using a pH meter 3510 (Jenway) according to the standard LVS EN 1132 - pH Determination of Fruit and Vegetable Juice.

### 3.RESULTS AND DISCUSSION

Quality of fruit products changes during technological process and subsequent storage depends on the alterations of cell wall components, in particular the pectin substances. Modification of pectin during processing is due to the action of endogenous pectin degrading enzymes, such as PME and PG. The synergic action of the two enzymes, both bound to the plant cell wall, yields short demethylated pectin chains, leading to softening of fruit and vegetable products and decrease of viscosity. The fruit juices can be considered biphasic systems, made up by a dispersing phase, called serum, in which the hydrosoluble components are dissolved, and a dispersed phase, called cloud, consisting of particles. The cloud phase, originating from plant tissue mechanically crushed in puree preparation, is constituted by solid particles with different size and shape. These particles, essentially made of polysaccharides and, to a lesser extent, of proteins and cell wall-bound enzymes, are maintained in suspension by the interaction with soluble pectin. During the juice extraction step, PME de-esterifies the pectin. The low methylesterified pectin may interact with calcium ions forming insoluble pectates, which co-precipitate with the pulp-particles, thus causing cloud loss. The degree of pectin methylation critical for this event is not yet well defined, due to the high heterogeneity of the pectic fraction in a complex system such as a vegetable juice.

The juice stability also depends on the charge and hydration of pulp-particles in the suspension, both affected by the action of PME. Therefore, PME is responsible for particle precipitation and cloud-loss of fruit juice. In order to prevent this phenomenon, PME must be inactivated. This is usually achieved by heat treatment, such as pasteurisation. It is widely accepted that this treatment is sufficient to inactivate PME, although the assay of the residual PME activity in the heat-treated juices is generally performed with the pH-stat method, which is not sensitive enough to detect weak enzymatic activity.

In fact, cloud loss of heat-treated juices often still occurs and its control is a serious problem in the food industry. Much attention has been dedicated to the chemical and physical factors affecting the phenomenon, while the biochemical aspects of the problem have been investigated to lesser extent. The finding of residual PME activity in the commercial juices of some fruits suggests the existence of extremely thermostable PME isoforms which survive the heat treatment and indicate that some production process may be further improved.

The affinity between PME and PMEI increases as pH decreases, in the range 7.5–6.0. It is conceivable that the stability of the PME/PMEI complex is higher at acidic pH usually found in fruit juices. The PME inactivation by PMEI could allow heat treatment of juices at temperatures lower than those needed for inactivation of highly thermostable PMEs. Such milder heat treatment could result in better flavor quality and higher vitamin content, thus producing high quality products (Giovanea A. et al, 2004).

Heat treatment of fruits is a crucial method of preservation. When the pectin substances in cell walls decompose, softening of wall cells and subsequently separation may happen. The divalent ions form cross-link between carboxyl groups of pectinic acid molecules, will enhance the firmness of the middle lamella and primary cell wall. But when monovalent ions are present the cross-link formation is stopped and consequently rigidity will decrease.

As plant tissue is heated, intracellular ions such as calcium and magnesium may come in contact and react with cell wall components, e.g. free carboxyl groups to create bridges that toughen the tissue so that it may withstand degradation during heating process. Another significant aspect in loss of firmness during the heating process is the pH.

The pH activity in apple mass was analyzed in our research and results are shown in Fig.1.

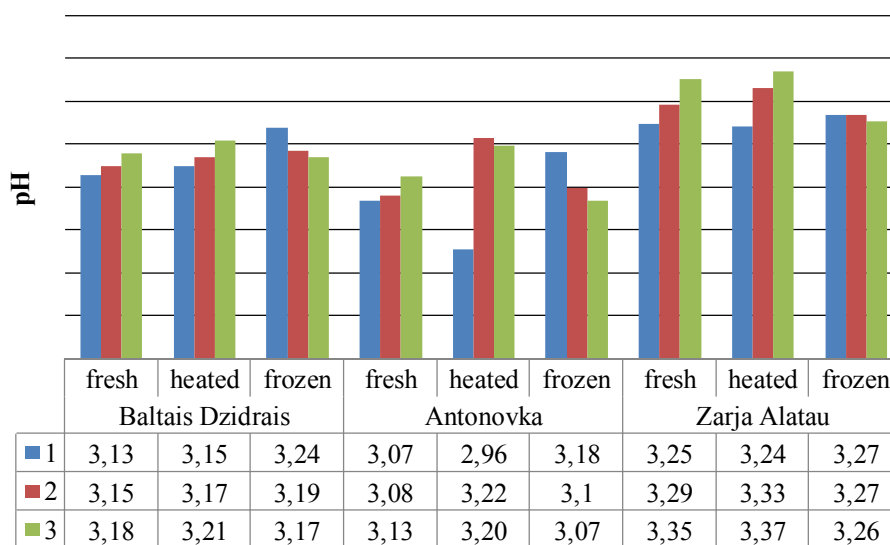


Fig.1. pH activity in fresh, heat-treated and frozen apple mass

“1”, “2”, “3” – storage time, weeks (fresh ‘Baltais Dzidrais’);

“1”, “2”, “3” – storage time, months (‘Antonovka’, ‘Zarja Alatau’, frozen ‘Baltais Dzidrais’)

The results of pH activity in apple mass indicate that pH increases during storage time but decreases in frozen condition. This could be explained by activity of acid in apples during storage time. Hungarian scientists in their researches have established increase of acidity in fruits and berries, emphasizing changes of flavor properties what is very characteristic for such picturesque berries as strawberries. In researches of apples it has to be considered that only sliced apples are suitable for freezing what cannot describe dispersion of acids in the whole fruit. The researches carried out enable description of pH activity in the whole fruit.

During freezing concentration of juice and pH changes take place. High water content in the product but sometimes also low pH value can increase efficiency of cold activity. Sugar, colloids and some other substances, which are added in small amounts, may reduce impact of cold activity. Therefore addition of sugar and pectin has to be performed after freezing in frozen condition. (Kampuse, 2006)

With the pH determination in deep-freezing the results depend largely on the duration of freezing and the time interval between thawing and pH measurement. A description is given of quick methods for the determination of sugar and acid contents in apples to be used in growing for fruit quality.

In an acid medium polysaccharides gradually hydrolyse till monosacharides. Raw apples generally contain a small quantity of starch, which gets wholly converted into sugar during the process of ripening. In this research (Fig.2) also soluble dry matter was determined in order to analyze sugar changes in apples during storage, heating and freezing.

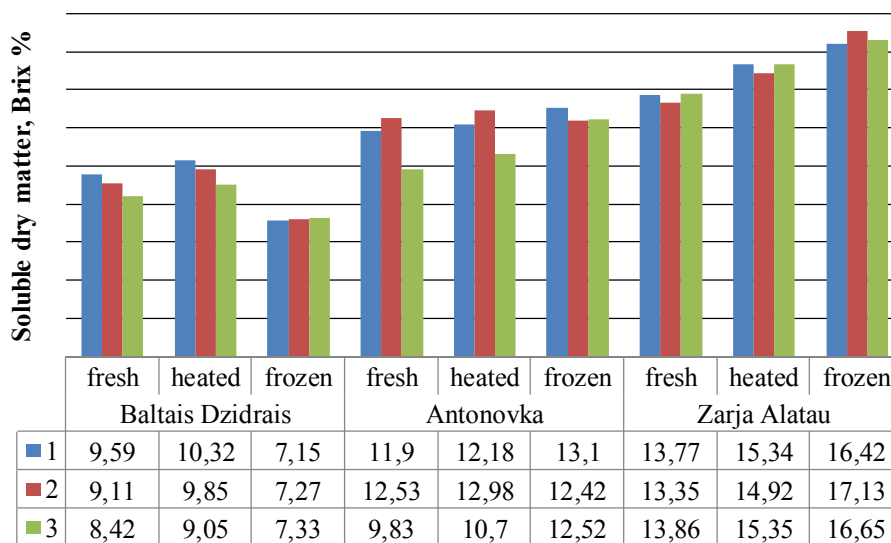


Fig. 2. Soluble dry matter in apple mass depending on storage time, heating and freezing

“1”, “2”, “3” – storage time, weeks (fresh ‘Baltais Dzidrais’);

“1”, “2”, “3” – storage time, months (‘Antonovka’, ‘Zarja Alatau’, frozen ‘Baltais Dzidrais’)

Research data indicate that soluble dry matter may either increase (‘Antonovka’, ‘Zarja Alatau’) or decrease (‘Baltais Dzidrais’) depending on variety. There is more soluble dry matter in fresh apples of the variety ‘Baltais Dzidrais’ than in frozen ones. Whereas there is less soluble dry matter in fresh apples of the varieties ‘Antonovka’, ‘Zarja Alatau’ than in frozen apples. This can be explained by the fact that ‘Baltais Dzidrais’ is a summer variety, harvest and storage time of its apples is short. The autumn-winter varieties ‘Antonovka’ and ‘Zarja Alatau’ are harvested unripe (because of climatic and storage considerations) and ripen in the warehouse reaching good edible or consumption maturity. Sugar content increases upon the ripening of apples, but when the apples become overripe, sugar content decreases. Research data indicate that apples of the variety ‘Antonovka’ become overripe during the third month of storage time, because their sugar content decreases. Whereas apples of the variety ‘Zarja Alatau’ at the third month of storage time reach good consumption maturity what is proved by sugar content in them.

In our research apples after harvesting were made in a mass to improve their quality after freezing and thawing. Various ingredients may be added to enhance the quality of frozen fruits. Water is drawn out of the cells by osmosis, which leads to a lower freezing point and a decreased fraction of ice at a certain frozen storage temperature. An extension of this is osmotic dehydration in which high concentrations of sugar solutions are used. By this approach, fruit cells may lose up to 40–70% of their water. The formation of extracellular ice concentrates the solutes in the surrounding aqueous phase, which in turn drives water from the cells. The higher concentration of intercellular solutes, coupled with the lessened flexibility of the frozen phase, produces cell and cell membrane damage. In addition, cell wall substances, in particular pectin, may be extracted or solubilized from the cell walls, reducing structural rigidity. Quick freezing results in smaller ice crystal size, less cell dehydration, and less cell damage.

The fruit variety may also be a factor in freezing performance. Immediate freezing of fruit after harvest can also improve texture. One of the best postharvest methods for fruits and vegetables is

freezing – process, required optimal conditions of technology. (Min Zhang, 2004) In addition, the correct choice of refrigeration technology enables maintaining sensory properties of fruits and vegetables (appearance, texture, etc.) to maximum, which enables the use of varied opportunities for freezing and after thawing it. This is potentially important for fruit and vegetable processing companies to use fresh or frozen fruits as basic in the market, as well as some other semi-finished products. Ice formation in food products takes place at relatively low temperature and products stored at the temperature -18 °C are not yet fully frozen. Products containing higher concentration of small molecular sugar at low temperature contain more unfrozen water because quick frozen carbohydrate solutions as well as most of biological products are characterized by non-equilibrium formation of ice crystals with its specific concentration of solution (Roos, 1995). Freezing time increases somewhere between linearly with or as the square of the shortest dimension for heat transfer. Hence, a lot of fruits are used for further processing, and fruit cut prior to freezing is often easier to cut and of better quality than fruit cut after freezing and thawing. (Kerr, 2007)

Many fruits are frozen to extend their availability throughout the year, and to further shelf life through lengthy distribution. In general, fruits have a less fibrous texture than vegetables and often suffer more textural changes during freezing and storage. (Skrupskis et al., 2008)

Freezing is used to preserve and maintain quality of many foods. Relatively low temperatures, the formation of ice as a separate phase, and the frozen concentration of dissolved substances contribute to conditions that limit the growth of microorganisms and preserve quality factors such as flavor and color. (Bing Li, 2002)

One critical quality factor influenced by freezing is food texture. Texture can be defined as those properties of food determined by the rheological and structural nature of the food and determined by the tactile senses. Many foods are thawed from the frozen state and eaten directly, or cooked before consumption. In some cases, the texture of the thawed material is close to that of the fresh and unfrozen food. In other cases, the texture may be changed by the freezing process and yet result in a thawed product that is still acceptable to consumers. (Kerr, 2007)

Many frozen fruits are destined for further processing, as for use in muffins, ice cream, yogurt, or jams. Hence large whole fruits do not freeze well. Fruits such as apples are made in a mass for freezing. Investigations show that storage in the place of growing decreases loss for 18% in average, but by using combination of natural and artificial cold the economic effect is much higher. Temperature is an essential factor in decreasing intensity of respiration.

Freezing is an ancient technology for preserving foods. Freezing halts the activities of spoilage microorganisms in and on foods and can preserve some microorganisms for long periods of time. Frozen foods significantly increase the storage life and maintain maximum product quality original figures.

As the main quality changes influenced by freezing can be mentioned color changes and jelly firmness or consistency changes. As a result of freezing jelly firmness essentially decreases therefore jellies made of frozen berries are with softer consistency. Freezing essentially decreases jelly firmness in products of those blackcurrant varieties which show good gelling abilities in fresh condition. Varieties possessing the most firm texture before heating produce the weakest jellies after heating. Thus it can be concluded that pectin in blackcurrant jellies is more sensitive to repeated gelling than to freezing. Freezing before heating does not influence substantially texture and floatability of jellies. Freezing essentially decreases the rate of berry color and sweetness. (Kampuse et al., 2005)

Transformation of pectin causes sediment formation in frozen berry juices and texture hardening of some berries. If berry juices are not sufficiently heat-treated, pectinesterase occurs in them. It takes away methyl radical from pectin molecule what causes occurrence of insoluble pectic acid, in particular, if the water contains calcium. Texture hardening of berries also is caused by esterase as the

formed pectin acid together with calcium contained in fruits produce calcium pectinate gel. This process is significant in long-term storage even at the temperature -18°.

Moreover, pectic acid forms in overripe fruits, therefore it should be provided, that fruits neither before harvesting nor during storage are overripe; products made from fruits containing less than 0.35% pectin substances, are not jellying. On the basis of worked out functional analysis of colloids, it is possible to optimize regulation possibilities of heat processes for different ripeness stages of apples. The fiber in apples, which is thought to play a major role in its lipid-lowering capacities, is not found in particularly high concentration (2–3 g/100 g), and soluble fibers such as pectin represent <50% of the fiber in apples. Nevertheless, it has been reported that this fraction probably contributes to the effects of apples on lipid metabolism. (Aprikian et.al, 2003)

There are data available in scientific literature, that total quantity of pectin substances in apples is 0.3 – 1.8%, pectin substances are encountered in the form of protopectin, pectin and pectic acid. In green, unripe fruits, pectin is in the form of protopectin, which is of higher density and firmness than pectin. Both under the impact of ripening and heat treatment, protopectin transforms into pectin, fruit jelly forms. (Kreichbergs, 2008) It is established that ripeness stage influences pectin quantity in fruits, but there are no data about pectin quantity changes in frozen fruits. In order to find out whether pectin quantity in frozen fruit mass differs from fresh apple mass, analyses were carried out and data compared of fresh, heated and frozen apple mass. Pectin quantity was expressed by part of galacturonic acid in apple mass mg/kg. Figure 3 shows the data of pectin quantity in researched apples.

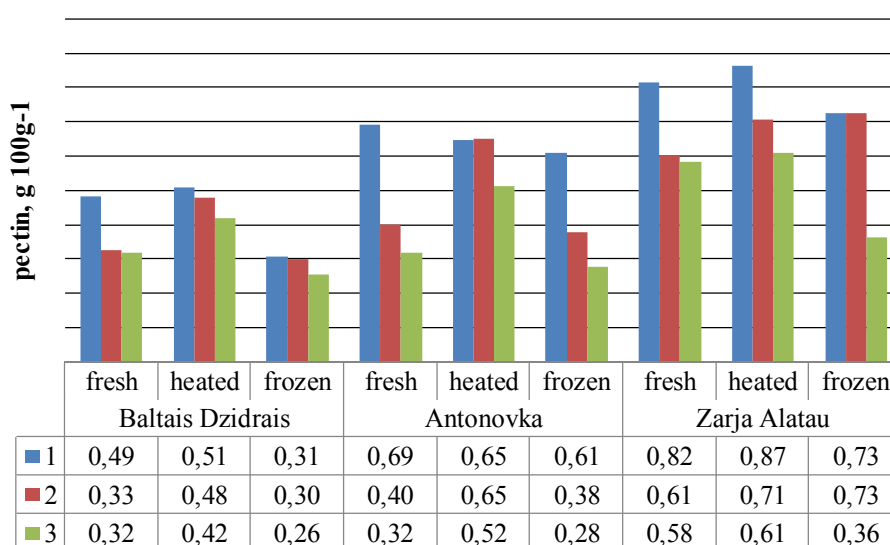


Fig.3. Pectin quantity in fresh, heat-treated and frozen apple mass

“1”, “2”, “3” – storage time, weeks (fresh ‘Baltais Dzidrais’);

“1”, “2”, “3” – storage time, months (‘Antonovka’, ‘Zarja Alatau’, frozen ‘Baltais Dzidrais’)

Results of the research indicate that upon ripening and heating pectin quantity decreases also in frozen apple mass. Pectin quantity during 3 months of storage time has less decreased in the apple variety

‘Zarja Alatau’ – only for 29.27%. The apple variety ‘Antonovka’ has the greatest loss of pectin, where pectin quantity during storage time has decreased for 53.62% and during freezing – for 54.1%. The heated apple mass of the variety ‘Baltais Dzidrais’ had the lowest loss of pectin (17.65%).

The least loss of pectin in frozen apple mass was produced by the apple variety ‘Baltais Dzidrais’ (16.13%). Pectin quantity in heated apple mass is higher than in fresh and frozen apple mass what is common to all apple varieties.

While considering composition and properties of 12 apple varieties with the aim to make purees of them it is established that the variety ‘Antonovka’ has the highest evaluation. Polish scientists have included it in the variety register among which is also the world famous variety ‘Jonathan’. Russian researchers have found out that apple pectin possesses heterogeneity.

Batisse studied pectin solubilization during ripening of cherry fruits. Pectin materials were isolated at specific stages during development. The proportion of alcohol-insoluble-material decreased on a fresh weight basis upon ripeness, but increased per fruit. Pectin substances were divided into water-, oxalate- and acid-soluble fractions. The oxalate-and water-soluble part increased as acid-soluble components decreased. Water- and acid-soluble fractions contained many more neutral sugar residues than the oxalate-soluble fraction. Changes in individual sugars indicated the major alterations were the degradation of galactan and the absence of arabinan solubilization in acid-soluble pectin substance. Size exclusion chromatography showed absence of pectin depolymerization during ripening, indicating that fruit softening does not depend on pectin depolymerization. (Batisse C. et al, 2006)

By fruit ripening under the impact of enzyme protopectinase, protopectin hydrolyzes and transforms into pectin, which flows from the intercellular spaces into protoplasm of cells. Therefore ripe as well as heat- treated fruits are soft, loose. The concentrated enzyme preparations are readily inactivated by normal pasteurizing temperatures (68°–70°C), but in juices the enzyme shows much greater stability. The significance of these findings is discussed in relation to the production of apple juice and pectin. The study has been made of the pectin-demethylating enzyme system in apple tissue and the level of activity compared with that in some other fruits. The apple varieties tested, show considerable differences in their pectase content. The enzyme shows a maximum activity at pH 6–6: over the pH range of the fruit the activity is much lower, but the enzyme can still produce appreciable effects. (Pollard and Kieser, 2006)

Effectively, pectin's structure binds with water in an acid environment. Sugar increases pectin's ability to gel, and affects the texture and consistency of jellies and jams as they cool and set. Note: sugar is not a key to the preservation of the jam - the sterile environment and acidity are more important (Kays S., 2004)

Pectin quantity in all fruits is generally higher when fruit is just barely ripe and reduces upon maturity from fully ripe to overripe. The process of ripening involves the breakdown of pectin, which softens the fruit upon ripening. Apples and crabapples (especially unripe ones) are good sources of pectin and are often used in making commercial pectin. Some commercial pectin is made from citrus peels. High-ester pectin at soluble solids content above 60% and a pH-value between 2.8 and 3.6, hydrogen-bonds and hydrophobic interactions bind the individual pectin chains together. These bonds form as water is bound by sugar and forces pectin strands to stick together. These form a 3-dimensional molecular net that creates the macromolecular gel. The gelling-mechanism is called a low-water-activity gel or sugar-acid-pectin gel. In low-ester pectin, ionic bridges are formed between calcium and carboxylic acid of the galacturonic acid. This is idealized in the so-called “egg box-model”. Low-ester pectin needs calcium to form a gel, but can do so at lower soluble solids and higher pH-values than high-ester pectin (apple pectin). During freezing concentration of juice and pH changes take place. High water content in the product but sometimes also low pH value can increase efficiency of cold activity. Sugar,

colloids and some other substances, which are added in small amounts, may reduce impact of cold activity. Therefore addition of sugar and pectin has to be performed after freezing in frozen condition. (Kampuse et al., 2005)

For each kind of product appropriate conditions have to be chosen for freezing, as well as the condition of products before freezing has to be taken into consideration in order to reduce to the minimum the harmful impact on their quality. Pectin solutions have high viscosity which depends on pH. Sedimentation or gel formation takes place at  $\text{pH} \leq 3$ . Gelatinization temperature of pectin ranges from 50 - 70°C where unrecoverable changes take place. During gelatinization pectin can absorb water mass and rise, as a result increasing suspension viscosity. (Morozovs A., 2008)

It is important to measure pectin, pH and soluble dry matter in fresh and frozen apple mass, in order to determine its potential gelling ability. Possibilities of adding ingredients, which are essential in dessert making, depend on gelling ability.

#### 4.CONCLUSION

1. The results of the research indicate that upon the ripening, pectin quantity decreases also in heated and frozen apple mass. Pectin quantity during 3 months of storage time has less decreased in the apples of the variety 'Zarja Alatau' (29.27%) and during freezing (16.13%) and heating (17.65%) - in the apples of the variety 'Baltais Dzidrais'. Apples of the variety 'Antonovka' have the highest loss of pectin where pectin quantity during storage time has decreased for 53.62% and during freezing - for 54.1%.
2. Changes of soluble dry matter and pH prove the freezing impact which has to be considered in technological process. The established differences in research of the varieties can serve as the base for the development of technological schemes and choice of proper freezing regimes.
3. If it is assumed that pectin ability to gel in apples decreases at 0.35%, then apples of the variety 'Baltais Dzidrais' possess gelling ability only one month after storage, and during freezing this ability decreases already just after freezing and heating. Gelling ability of the apple mass of the variety 'Antonovka' is maintained for 2 months after storage and freezing. The apples of the variety 'Zarja Alatau' have the most stable pectin quantity which gelling ability is maintained for 3 months after storage in fresh, heated and frozen condition. Pectin quantity in heated apple mass is higher than in fresh and frozen apple mass what is common to all apple varieties.

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