FREEZEWAVE – Innovative and low energy microwave assisted freezing process for high quality foods

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ABSTRACT

FREEZEWAVE project proposes a highly innovative technique which showed that a small amount of emitted microwave energy combined to a slow freezing rate is able to refine ice crystal size in frozen meat. FREEZEWAVE project aims at expanding & optimizing the concept to several foods and also at designing industrial equipment. Herein, the structure and the directions of the project as well as the initial results of this study and the preliminary results of the previous study will be illustrated. More specifically, the application of microwaves during cooling the samples caused oscillated decrease of temperature and had a significant impact on the crystallization process as the degree of supercooling was decreased circa 92% under the tested conditions. The meat microstructure evaluation showed a 62% decrease in the average ice crystal size when samples were frozen under a microwave field as compared to the conventional freezing process.

1. INTRODUCTION

The freezing process of food matrices is affected by their dominant constituent which is water. The final quality of the frozen product depends on the phase transition or the crystallization process of changing water into ice. The size of the ice crystals is critical for the final quality of the frozen food as it can cause irreversible damage to the cellular structure which in turn degrades the texture and colour of the product. For this reason, several emerging technologies have been developed in order to control the crystallization process and to improve the rate of ice crystal formation and growth. Most of these technologies take advantage from the physical properties and the characteristics of the water molecule.

Water molecules react when electric or magnetic fields try to disturb its tranquility (Orlowska *et al.*, 2009). Electrical and magnetic disturbances are factors that could rearrange the hydrogen-bonded network of water. Recently, one study on the application of an external static electric field to freezing of real food system has been published (Xanthakis *et al*., 2013). These results showed that during the freezing of pork tenderloin in the presence of high voltage static electric field (electrofreezing), as a result of controlled nucleation, the damage to the meat microstructure was significantly reduced and the quality of the frozen product improved.

Concerning the electromagnetic effects on water, Chaplin (2013) reported that the presence of electromagnetic fields (EMF) attempt to reorient the water molecules and some hydrogen bonds of the network may be broken. The influence of electromagnetic radiation on water was reported to result from the electrical rather than the magnetic impact. As described before, electric fields are more efficient in changing the conformation of water network due to the intrinsic electric dipole moment of water molecule, while much stronger magnetic fields are required in order to exert the same force. Furthermore, Chaplin (2013) suggested that lower frequency microwaves (915 MHz vs 2450 MHz) (MW) or radiofrequency radiation (RF) and even extremely low frequency fields (ELF) (3 Hz – 300 Hz) have significant and lasting effects on liquid water. There are claims that water shows memory effects on electromagnetic radiation due to the long lifetime that these effects seem to have. Although several studies have been conducted regarding the impact of electromagnetic fields on liquid water, just a few have been carried out concerning the application of such fields during the phase change of water (Xanthakis *et al*., 2014a).

Jackson *et al*., (1997) evaluated the effect of coupled interaction of MW radiation and cryo-protectant on cryopreservation biomaterials. They developed an experimental setup which was able to cool rapidly by plunging the samples into liquid nitrogen while simultaneously irradiating it with a powerful coherent field. The samples consisted of aqueous solutions of ethylene glycol in various molarities. Their results indicated that ice formation was significantly influenced by MW radiation. The authors suggested that the combined use of MW radiation and a cryo-protectant could be a potentially interesting approach to control ice formation in cells and tissues in order to improve vitrification of these biomaterials for long-term cryopreservation. Although these results were promising, limited investigations have been conducted in this area since then.

Anese *et al*., (2012) carried out another study on the application of radiofrequency waves during freezing of meat. A pilot scale RF equipment was used while liquid nitrogen was flowing in a chamber in order to provide cryogenic freezing conditions to their samples. Although RF pulses with various voltages were applied, a meat sample was completely frozen at 2 kV. Although no data were presented on ice crystal size, neither on time-temperature history, the histological micrographs seem to indicate that the ice crystal size decreased.

In our preliminary study a novel experimental setup was designed and developed for the application of microwave radiation during freezing (Xanthakis *et al*., 2014b). The influence of microwave assisted freezing on a food system has been considered.

FREEZEWAVE is the continuation of this innovative technology by an integrated and comprehensive approach. More specifically, FREEZEWAVE aims to:

a) develop innovative and low energy demanding freezing process for high quality foods
b) develop new models and scientific knowledge on freezing under oscillating electrical disturbances
c) set up a number of protocols and knowledge on innovative concepts of freezing
d) assess the effect of microwave waves on reduction of the size of ice crystals and overall quality of frozen foods
e) develop and optimize the process in batch conditions to different food systems, such as sauce (emulsion), vegetable, meat & ready to eat meal.
f) scale up the process to industrial scale in the case of batch & conveyor system

2. MATERIALS AND METHODS

2.1 Microwave assisted – freezing equipment development

The prototype experimental set up consisted of a heat exchanger with continuous flow circulation of a fluid using an external cooling bath, a domestic microwave oven and a real time-temperature measurement system connected to a PC. The sample holder was made from a block polystyrene foam (Dow Chemical Co.). The heat exchanger was constructed by machining an Ertalon block (Figure 1a). The meat sample was installed in a cylindrical cell located in the center of the sample holder (Figure 1b). The upper surface of the heat exchanger was made of tempered glass and was used as the heat exchanging surface between the sample and the heat exchanger. A cover made of polystyrene was covering the sample on the top of the sample holder in order to avoid heat exchange between the sample and the cavity of the oven. The heat exchanger was placed at the middle height and at the central location of the microwave oven (Danby DMW608BL – 700 W, 2.45 GHz) cavity, while 4 glass flasks filled with 250 mL distilled water each were placed at the four corners of the cavity. The cooling bath (HAAKE PHOINIX II Thermoelectron Corporation) was connected with the input and the output of the heat exchanger by plastic insulated tubes. For the real time measurement of temperature of the sample during freezing an optical fiber was placed in the center of the sample and connected to an optical fiber station (OSB system Fiso) which in turn was connected with a PC provided with the proper data logging software (FISOCommander, Fiso Technologies Inc.).

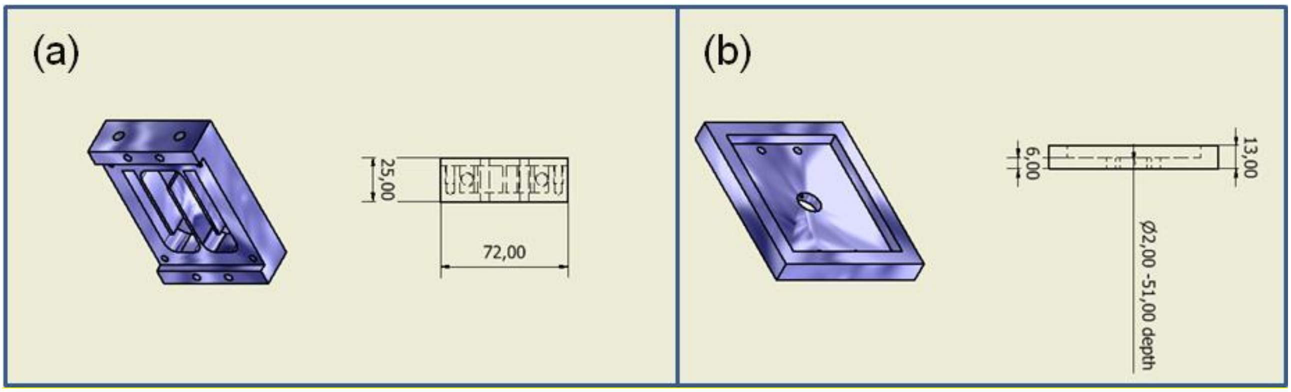

Figure 1. (a) Heat exchanger and (b) sample holder. The units of all the referred dimensions are mm.

2.2 Preparation of meat sample and freezing procedure

Fresh pork tenderloin was purchased from a local supermarket (Maxi, Montreal, Quebec, Canada) on the first day of distribution. Cylindrical pieces of pork tenderloin were carefully cut parallel to the fibers of the muscle. The radius of the cylindrical sample was 7.5 mm and height 6.0 mm. The weight of each sample was measured giving an average mass equal to 1.48±0.08 g. Each sample was installed into the cylindrical cavity of the sample holder with the fibers oriented perpendicular to the refrigerated surface of the heat exchanger. The extremity of the optical fiber was centered in the cylinder at mid height. Then the sample was cooled and equilibrated at 1ºC. The temperature of the cooling bath was lowered to -30 ºC with a cooling rate of 2 ºC/min and flow of 6 L/min.

2.3 Preparation of samples for microstructure analysis

Both fresh (unfrozen) and frozen meat samples were prepared for microstructure analysis. At least 4 independent repetitions were carried out under each microwave power level. The samples were fixed using Carnoy solution (60% absolute ethanol, 30% chloroform and 10% glacial acetic acid, v/v, purchased by Merck KGaA in analytical purity) and further processed according to the protocol followed by Xanthakis *et al*. (2013).

2.4 Determination of the freezable water content

The latent heat corresponding to the ice-water transition in the tenderloin pork meat was determined using a μDSC 7 EVO DSC (Setaram) device connected to a cooling bath. The initial temperature in the cell was -45 ºC and a scan at 1 °C/min was programmed up to a final temperature of 20 °C. 10 min dwell isotherms were programmed at the initial and the final temperatures. The reference cell was an empty cell similar to the one containing the meat sample. The samples of the meat were weighed (100 – 150 mg) in the sample's DSC cell. The programming and the elaboration of the spectra were performed with Data Acquisition (version 4.1D - TA Instruments) and Calisto Processing (version 1.065 - Setaram) software packages. The measurement of meat dry matter was carried out with the dehydration of pre-weighed samples for 48 h in an oven tuned at 104 ºC. The results were acquired as an average of 6 repetitions from different batches of fresh pork meat. The percentage of freezable water (FW%) was calculated using the area of the integrated endothermic peak at the range of 0 °C. The endothermic peak arises from the phase transition of ice into water. Eq. (1) was used in order for the freezable water of the meat to be estimated.

$$FW\% = \frac{Q \cdot 100}{H_f \cdot m_s} \tag{1}$$

FW = percentage of freezable water [%], Q = enthalpy [J], H_f = heat of fusion ice-water [333.50 J· g^{-1}], m_s = mass of the sample [g].

2.5 Statistical analysis

The results of this study are presented as the mean values accompanied with their standard deviations of at least four independent experiments. One factor analysis of variance (ANOVA) was performed. Differences between means were considered statistically significant at $p<0.05$. The software which was used for the statistical analysis was Statgraphics Centurion XVI, Version 16.1.17, (Warrenton).

3. DISCUSSION

3.1 Freezing under Microwave radiation

The present study is focused on the damage caused by the ice crystals to pork meat tissues under different power levels of microwave radiation during freezing. Since this was the first study of microwave-freezing of food, at least to our knowledge, several trials and tests were carried out prior to finalizing the described process.

This study was focused on 4 (40%), 5 (50%) and 6 (60%) power level settings of the microwave oven because at lower levels no influence appeared on the ice crystal sizes while higher power was restricted due to heat transfer limitations. For determining the influence of the microwave radiation during freezing, this study investigated the temperature profiles of the developed freezing processes.

Representative real time temperature profiles during freezing under microwave radiation are shown in Figure 2. When the sample was frozen without the application of microwave radiation the temperature curves were smooth, while under microwave radiation the oscillated temperature appeared to decrease in steps.

The overall freezing time was longer in the MW-freezing conditions as compared to the conventional freezing and it increased in tandem with the increase in power level. Additionally, the freezing rates between the different power levels of microwave radiation were compared between the temperature range from -5° C to -10 °C. As reported earlier (Le-Bail *et al*., 2008), in such food matrix the major amount of ice is formed in this temperature range. The statistical analysis showed that there was a significant decrease of the freezing rate when the three levels of microwave power were applied compared to the conventional freezing. Moreover, the degree of supercooling dropped significantly when a power level of 40% microwave radiation was applied. The degree of supercooling was further decreased when the microwave power level was higher.

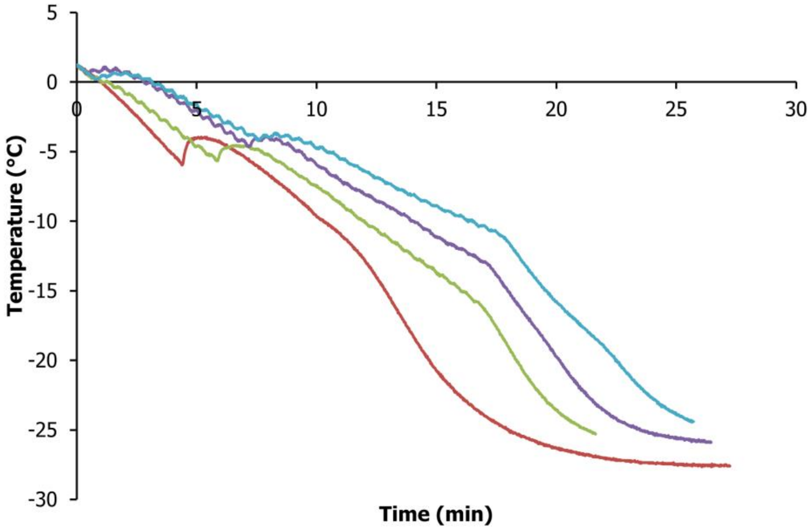

Figure 2. Real time – temperature plots obtained during conventional freezing (red curve) and under different power levels of microwave radiation of pork tenderloin samples (40% - green curve, 50% - purple curve, 60% - blue curve).

It is known that the interactions between nucleation and crystal growth are of great importance as they are responsible for the size, distribution and morphology of ice crystals during freezing. More specifically, several studies have suggested that rapid crystallization rates and high degree of supercooling promote the formation of numerous ice nucleus and small sized ice crystals. Further, in both homogeneous and heterogeneous nucleation processes, the nucleation rate has a dramatic increase with the increase of the degree of supercooling (Burke *et al*., 1975; Fletcher, 1970; Chevalier *et al.*, 2000), and the size of the ice dendrites are dependent on the degree of supercooling (Teraoka *et al*., 2002).

The proposed process takes advantage of the fact that water molecule can be influenced by the presence of electromagnetic disturbances. Generally, nucleation process is very sensitive and it can prematurely occur when external disturbances are applied. Furthermore, it seems that the electromagnetic disturbance which was applied during the presented process induced a premature nucleation as the degree of supercooling was significantly decreased. Moreover, as it can be assumed from the real time temperature curves, the periodically microwave electromagnetic radiation transmitted energy to the sample which was transformed into heat. At the same time, the heat exchanger absorbed the heat from the sample continuously. This combination of double heat exchange resulted in the freezing of the sample through oscillated decreasing temperature.

3.2 Microstructure analysis of the frozen samples

In Figure 3 micrographs of representative images of pork tenderloin samples after freezing under different microwave power levels are presented. In these images transversal cuts to the fiber direction of the samples are illustrated. The tissues of the pork tenderloin muscle are shown in red while the voids (white zones) represented the location of the ice crystals that were formed in the microstructure during freezing. From a qualitative point of view it can be assumed that the damage to the tissues of the meat is higher with the conventional freezing procedure (Figure 3a) than with the application of microwave radiation during freezing (Figure 3b, c & d).

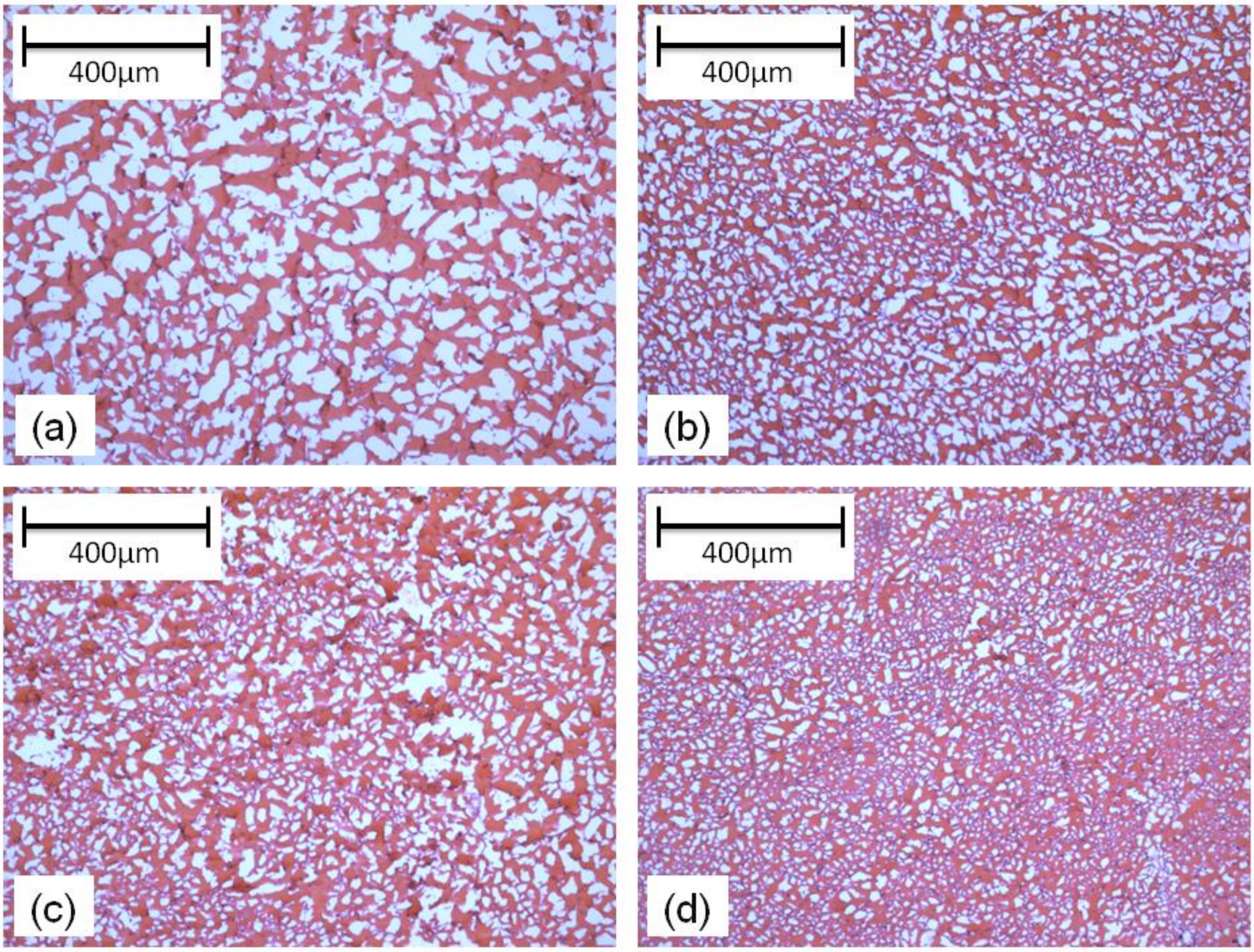

Figure 3. Micrograph images of frozen pork tenderloin transversal cuts under different levels of microwave power radiation. (a) 0 % (conventional freezing), (b) 40%, (c) 50% and (d) 60%.

The microstructure of the meat during freezing was quantified to evaluate the microwave radiation effects. For this purpose, a microscopic 2D image analysis was used to determine the size of the ice crystals as well as to obtain some information about the morphology of the ice crystals. The equivalent circular diameter (EqD) was measured for the estimation of the ice crystal growth alterations under different experimental conditions. EqD is defined as the diameter of a circle that has the same area as each crystal 2D footprint. A significant statistical change ($p<0.05$) in the ice crystal mean EqD was achieved when the meat samples were frozen under all tested power levels of microwave radiation. Although with an increasing microwave power level the average EqD didn’t show a significant decrease, at 60% power microwave level the ice crystals showed around 62% decrease of their average diameter compared to the conventionally frozen samples. These effects can be correlated with a recent study on meat freezing carried out under the high frequency - radiofrequency waves (Anese *et al*., 2012). Both confirmed a decrease in the size of the ice crystals formed during freezing, although that the freezing conditions and radiation frequencies were different for the two methods. As mentioned earlier, during conventional freezing, two parameters that are critical for minimizing the ice crystal size are the degree of supercooling and the freezing rate. However, in these terms the results of this study do not follow the general theory of conventional freezing. The presented method, although it was followed by a lower degree of supercooling and slower crystallization process, nevertheless led to a significant decrease of the formed ice crystal size. The impact of microwave application during freezing on the ice crystal size seemed to be related to the oscillation of temperature at both stages of nucleation and crystal growth. The limited oscillation of the temperature during the genesis of the ice nuclei and crystal growth may have been responsible for instantaneous recurring melting and regeneration of ice crystals which in turn prohibited the crystal growth and led to the numerous and smaller ice crystals which were observed. In another recent study, the size of the ice crystals was also reduced when meat samples were frozen under high voltage static electric field (Xanthakis *et al.*, 2013). In that study, it is reported that the presence of static electric field induced nucleation and decreased the degree of supercooling during the freezing process. Although that the underlying physical phenomena are not clear, seems that external factors such as static electric fields and electromagnetic radiation can influence the crystallization process without being aligned with the general theories of the conventional freezing.

4. CONCLUSIONS

During the aforementioned study a novel and innovative food freezing process was introduced. The results acquired regarding the freezing of pork tenderloin under the presence of microwaves revealed that, apart from controlling the nucleation temperature, the final quality of the frozen product also may be improved as the damage of the meat microstructure was significantly improved under the tested conditions. This first achievement demonstrates an effectiveness of the microwave radiation in controlling ice crystal size. Further investigation is needed in the direction of the underlying heat transfer phenomena of the process and application of this innovative freezing process to other food systems would be beneficial. Towards this direction, FREEZEWAVE project comes to contribute and integrate this process by investigating in depth the underlying phenomena, building an improved lab-scale equipment as well as a conveyor microwave assisted freezing system for industrial purposes.

5. ACKNOWLEDGEMENTS

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