Bioresistance of thermally-modified *Populus tremuloides* (North American Aspen) wood against four decay fungi

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Abstract

A study was carried out to investigate the effect of thermal treatment on biological resistance of *Populus tremuloides wood* against four decay fungi, including *T. versicolor*, *P. placenta*, *G. trabeum* and *C. puteana*. The weight loss data showed that degradation of untreated wood by the white rot fungus *T. versicolor* resulted in higher weight loss (57.1%) compared to those by brown rot fungi *G. trabeum* (21.9%), *P. placenta* (36.7%) and *C. puteana* (7.1%). When this species was heat treated at 220°C, the weight loss was reduced to less than 10%. Increasing temperature and the holding time appeared to affect the resistance against *T. versicolor*, *P. placenta and G. trabeum* attacks, but the effect on the resistance against *C. puteana* was not significant. The humidity during thermal modification affected the degradation of wood against *T. versicolor* attack, but it did not have a significant effect the other fungi.

Keywords: Biological durability, Thermally-modified wood, High temperature wood treatment, Thermotransformation, *Populus tremuloides*, decay fungi, white rot fungi, brown rot fungi.

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Introduction

Biological durability is a major factor in the wood industry in the selection of wood species for certain applications. Availability of suitable choice avoids unnecessary expenses of replacing the damaged parts and reduces its impacts on the remaining forests. One third of the circumpolar boreal forest in the Northern Hemisphere is located within the borders of Canada and *Populus tremuloides,* used in this study is an endemic species of this forest. Consequently, adding value to this species is very much important for the economy of Quebec and Canada.

Many organisms can deteriorate wood. Among the micro-organisms responsible for wood degradation, fungi form a dominant group in terrestrial ecosystems (Schwarze et al. 2000). These organisms can recognize the natural polymers (cellulose, hemicellulose and lignin) in the cell walls of wood as source of food that they will metabolize by the action of enzyme systems (Paes et al. 2004).

In order to increase the biological durability of wood, several methods have already been the subject of different studies, such as using heavy metals (chromium, copper, and arsenic), creosote, and a variety of organic compounds. But these methods have limitations in their application due to their toxicity on the human health and the environment. Thermal modification is an alternative to wood preservation, which may result in environmental benefits (Homan et al. 2000; Tjeerdsma et al. 2000; Calenego et al. 2010; Metsä-Kortelainen et al. 2011). Thermal modification changes the chemical composition of wood, thereby altering the appearance, physical and biological properties of the wood. One of the main objectives of thermal modification is to increase the

biological durability and dimensional stability of wood. The biological durability of thermally-modified wood has been widely studied (Tjeerdsma et al 2000; Kamdem et al. 2002; Hakkou et al. 2006; Boonstra et al 2006; Welzbacher and Rapp 2007; Lekounougou et al. 2009). Different authors have tried to explain the improvement in durability of thermally-modified wood (Tjeerdsma et al. 1998; Kamdem et al. 2002; Welzbacher and Rapp 2007). According to Weiland and Guyonnet (2003) there are three main reasons for this improvement: thermal modification of wood stimulates the formation of new substances which act as biocides; the treatment chemically modifies the wood substrate so that it cannot be clearly identified by the fungus; and it degrades the hemicelluloses, the main source of food for the fungus. Hill (2006) mentioned that low cell wall moisture content and the loss of OH group in the polymer components of the cell wall could potentially affect the ability of enzymes to metabolize the substrate.

Data provided by Canadian Forest (2007), the largest private group working on the Canadian forest, and FPInnovations-Forintek (2010), a non-profit research institute on wood in the world, showed that forest industry in Canada has over 7,000 operations, which provide nearly 400,000 jobs in wood products, paper and manufacturing, logging and forest services. Canada export about $40 billion a year of wood products and is the world's largest producer and exporter of newsprint. It is estimated that 50% of the wood harvested in Canada comes from the boreal forest. *Populus tremuloides* is an important commercial species present in Canadian boreal forest and throughout Quebec. This specie is the most popular in North America; it is used in the industry of wood products (pulp, plywood and building). The possibility of total *Populus tremuloides* in Quebec is 6,000 000 m^3/year, with 13.3% in the Saguenay-Lac-St-Jean. But this species is sensitive to

decay-fungi. Thermal modification of *Populus tremuloides* adds value by extracting wood humidity, and improving characteristics such as durability, stability, attractiveness and environmental benefits. This wood can be used in both indoor and outdoor products, such as floors, kitchen, bathrooms, door, swimming pools, garden, furniture etc.

The biological durability of thermally modified wood has been assessed in many investigations using laboratory tests. However, the fungal resistance of thermally modified *Populus tremuloides* wood of Canadian forests has not been studied in detail.

The aim of this study is to assess the effects of thermal modification of *Populus tremuloides* and its process parameters on the biological resistance of *Populus tremuloides* against four basidiomycetes fungi attacks. The reasons for improved resistance of thermally-modified wood against fungal attacks are also discussed from molecular view point.

Materials and Methods

Preparation of samples

Populus tremuloides wood was sawed in a local mill at Saguenay-Lac-Saint-Jean (Québec, Canada). The boards were dried in air. The final humidity of the boards was about 5-17%. Then, they are thermally modified. After, wood samples required for fungal decay tests were prepared as explained below. The sapwood part of the wood was used for these tests.

Thermal Modification Process

The prototype furnace of University of Quebec at Chicoutimi (UQAC) was used for thermal modification of *Populus tremuloides*. Dimensions of the boards were 0.015 m x 0.045 m x 2.44 m in the radial, tangential and longitudinal directions, respectively. In this furnace, propane was used as gaseous fuel and the wood was modified thermally in a non-oxidizing environment composed of hot combustion gases (CO_2 and H_2O). The wood samples were treated at different maximum temperatures (180ºC, 190ºC, 200ºC, 210ºC, 220ºC), heating rate (10, 15 ºC/h), holding times (0, 1, 3 h) and gas humidity (0, 100 g water vapor/m^3 dry gas). Holding time refers to the period where wood boards are maintained at constant maximum treatment temperature. Thermal modification conditions are shown in Table 1. One parameter was changed at a time, keeping other parameters constant during the tests. A detailed description of the process was published by Poncsak et al. (2006).

Resistance against decay fungi

Four species of decay fungi, *P. placenta* (FTK120E), *G. trabeum* (FTK47D), *C. puteana* (FTK9B) (brown rot) and *T. versicolor* (FTK105D) (white rot) were tested during this study. These fungi were obtained from the mother culture supplied by FPInnovation FORINTEK, Quebec, Canada. The reisolation of the strains was done in petri dishes containing 30 mL of malt agar substrate. Cultures of 1 cm by 1 cm were placed in petri dishes. The growth was observed for seven days after inoculation. Wood samples with dimensions of 0.015 x 0.005 m 0.0035 m in the radial, tangential and longitudinal directions, respectively, were prepared. The treated and untreated wood samples were dried for 48 hours at 103ºC±1 in a furnace. Before studying the effect of fungi, the initial

dry masses (WL-I) of the wood samples were determined. The fungal decay test was done following EN-113 (1986) standard, with the exception of samples size which was reduced to accelerate fungal decay and reduce the duration of tests from 16 to 12 weeks. At least 60 mini blocks were used for each treatment.

Preparation of culture medium

A malt agar based substrate was used as nutrient medium for cultivation of fungi in petri dishes. It was prepared by dissolving 40 g malt extract and 30 g agar in 1L distilled water. The medium was sterilized for 30 minutes at 121ºC±1. 20 mL of agar was poured into sterile petri dishes to grow the fungi. A disc (about 5 mm diameter) of fungi was cut and placed in petri dish and incubated in a climatic chamber (Conviron) at 22ºC ± 1 and 70 % ± 4 relative humidity (RH) for two weeks. The petri dishes were found to be completely covered by the mycelium after two weeks. The treated and untreated wood samples, previously sterilized at 103ºC, were placed into petri dishes in the presence of four fungi *P. placenta*, *G. trabeum*, *C. puteana* and *T. versicolor* and incubated for twelve weeks. Every two weeks, the wood blocks were recovered and the mycelium was carefully scraped using a scalpel. Finally the blocks were placed in a furnace at 103ºC until their mass stayed constant. Three sets of each wood sample, both heat-treated and untreated, were placed in different petri dishes. Each experiment was carried out three times to ensure the reproducibility of the results. The mass was recorded as final mass (W_{L-F}). The percentage weight loss was calculated as follows:

$$\text{Weight Loss (\%)} = 100\ (W_{L-I} - W_{L-F})/W_{L-I}$$

where W_{L-I} is the initial mass of the sample (g).

The moisture content (MC) of wood blocks was determined after fungal decay tests using the following formula:

$$MC\ (\%) = 100\ (W_C - W_D)/\ W_D$$

where W_C is the wet mass after fungal attack (g) and W_D is the dry mass after fungal attack (g).

Results and discussion

The weight loss of *Populus tremuloides* wood due to attack of four decay fungi are summarized in Table 2. The results indicated that the white rot fungus *T. versicolor* was the most virulent compared to brown rot fungi *P. placenta*, *G. trabeum* and *C. puteana*. The weight loss data also revealed that *Populus tremuloides* can be classified as slightly resistant or non-resistant against *T. versicolor* fungus (57.1%). The same specie of wood can be placed in the class of moderate resistant against *P. placenta* (36.7%), resistant against *G. trabeum* (22.1%) and highly resistant against *C. puteana* (7.7%). The classification was made in accordance with the publication of Kartal and Ayrilmis (2005) which indicated that an average weight loss of 25%-44% was moderately resistant to decay fungi, a weight loss of more than 45 % was slightly resistant or non-resistant, and a weight loss of less than 10% was highly resistant. These variations can be explained by the difference in the lignin content of hardwood and softwood that can play a significant role in protecting natural wood. Presence of syringyl lignin in hardwood and relatively lower lignin content reduces the natural protection ability against fungi compared to that of softwood which contains guaicyl lignin (Daniel 2003). On the other hand, white rot

fungi had an enzyme system that can degrade the polysaccharides (cellulose and hemicelluloses) and lignin, while the brown rot enzyme system attacks the cellulose and hemicelluloses and causes slight damage to lignin (Highley 1999, Ten Have and Teunissen 2001). Other authors have also obtained similar results with the other wood species (Paes et al. 2004, Calenego et al. 2010).

Table 2 shows the effect of temperature during thermal modification of *Populus tremuloides* on the bioresistance against four decay fungi after 12 weeks of incubation. The results showed that the weight loss of wood decreased gradually when the treatment temperature was increased up to 220ºC for all the fungi tested in this study. Thermally modified *Populus tremuloides* at 220ºC was highly resistant to fungi *G. trabeum*, *P. placenta*, *C. puteana* and *T. versicolor*, and their mass losses were less than 10%. It can be seen from Table 2 that the weight loss after twelve weeks of exposure of untreated *Populus tremuloides* to *T. versicolor* was about 57.1% compared to 9% loss obtained for thermally modified wood at 220ºC. The reduction in percentage for treated wood was around 84.2% (Table 2) compared to that of untreated wood. Similar results were obtained with *P. placenta* with a weight loss 36.7% compared to weight loss of 0% for thermally modified wood at 220ºC. The reduction in percentage was around 100% (Table 2). These results are consistent with previous studies for other wood-fungi systems (Tjeerdsma et al. 2000; Kamdem et al. 2002; Weilland and Guyonnet 2003; Boonstra et al. 2006; Hakkou et al. 2006; Calenego et al. 2010).

During thermal modification of wood, there are chemical alterations of the cell polymers which are responsible for preventing fungal attacks and increasing wood durability. Another reason for the observed improvement in resistance against fungi attack of heat-

treated wood might be reduction in the cell wall porosities of wood that can prevent the penetration of fungal hyphae and their enzymes into the cell cavities or micropores of the cell walls. Several reports indicated that thermal modification of wood altered its chemistry, making it more hydrophobic and an unknown substrate for fungal enzymes, thereby making it difficult for fungal enzymes to attack the wood. Thus, increasing thermal modification temperature of wood can cause a series of chemical changes in the cell wall polymers (Garote et al. 2001, Bami and Mohebby 2011). In the literature it is known that hemicelluloses are the most thermo-chemically sensitive components of wood (de Groot et al. 1998, Bourgois and Guyonnet 1998). Any time, during heat treatment process, the cellulose microfibrils that are embedded by the hemicelluloses matrix are degraded with lignin modification (Levan et al. 1990, Kocaefe et al. 2008, Nazerian et al. 2010). All these chemical modifications could probably lead to non-recognition of the substrate by the enzyme system of decay fungi and cause a reduction in weight loss of wood by brown and white-rot fungi (Weiland and Guyonnet 2003). Lekounougou et al. (2008) showed a correlation of hydrolytic activities (cellulases and hemicellulase), involved in the hemicellulose and cellulose degradation, with weight loss of heat-treated and untreated beech degraded by white rot fungus *T. versicolor*. Hemicelluloses are degraded at low temperatures and transformed into acetic acid, formic acid, furfural, polyoses etc. As the temperature rises, the chances of occurrences of those compounds increase (Tjeerdsma and Militz 2005**,** Boonstra and Tjeerdsma 2006). Most of the transformed compounds, such as furfural, are toxic for fungi. These compounds prevent microorganisms from being active in the wood, or they can act as biocides (Kamdem et al 1999). The crystallization of the cellulose can also be another reason for preventing

fungal attacks especially by brown-rot fungi. These fungi prefer to attack the amorphous regions in the cellulose (Yildiz and Gümüşkaya 2007**,** Gonzales et al 2005**,** Green et Highley 1997). The condensation reactions of lignin as well as the appearance of new chemical bonds in the wood during thermal modification would cause changes in chemistry of lignin, making the substrate not distinguishable for peroxidases like lignin peroxidases (Weiland and Guyonnet 2003, Tjeerdsma and Militz 2005, Hakkou et al. 2006).

With increase in temperature of thermal modification of *Populus tremuloides* the resistance of the thermally modified wood improved against *T. versicolor* and *P. placenta* fungi, but little effect was observed for *C. puteana* (Table 2). This fungus appeared to cause some damage to the thermally modified wood. In the case of *G. trabeum*, there was an improvement in resistance with increase in treatment temperature even if the weight loss of the control was not significant.

Figure 1 shows the effect of holding time (exposure time) on the weight loss of thermally modified *Populus tremuloides* at 210ºC after 4, 8 and 12 weeks of incubation with *T. versicolor*, *P. placenta* and *G. trabeum* fungi. The results showed that the resistance against *T. versicolor* and *G. trabeum* decay fungi (Fig. 1a-b) improved for a longer holding time (3 hours) compared to that for a shorter holding time (1 hour). In the case of *P. placenta*, for 4 to 8 weeks of incubation, the efficiency of the length of holding time on the resistance against the fungi appeared to be similar with other results (Fig. 1c). On the contrary, after 12 weeks of incubation longer holding time seemed to have a limited effect on the resistance compared to that of shorter holding time (Fig. 1c). For 1 hour and 3 hours of holding times the weight losses were 19.4% and 16.1% respectively. The

thermally modified wood exposed to *T. versicolor*, *G. trabeum* and *P. placenta* was clearly affected even after an increase in holding time. Changes in structure of substrates (cellulose, hemicelluloses especially) due to long holdings time might contribute to the improvement of resistance. It is known that hemicelluloses are the first to degrade when the temperature increases. A longer holding time causes longer exposure to high temperature (210ºC), therefore, increases hemicelluloses degradation and initiates additional chemical reactions which may result in increase in resistance of thermally modified wood against fungal attack.

The heating rate had a limited effect on the resistance of wood against *T. versicolor* and *P. placenta* fungi (Figure 2). However, in the case of *G. Trabeum*, increasing heating rate seemed to improve the resistance of heat-treated wood against decay. If the heating rate is high, wood reaches higher temperatures quicker and might still contain some water. Presence of water has an effect on thermotransformation reactions, hence, on composition of wood (Poncsak et al. 2006).

Figure 3 presents the effect of gas humidity on the weight loss of thermally modified wood at 210ºC for 4, 8 and 12 weeks of exposure to *T. versicolor*, *G. trabeum* and *P. placenta* fungi. The results showed that the presence of humidity during heat-treatment of wood had a slight effect on the fungal degradation of wood. The weight losses were slightly significant for *G. trabeum* and *P. placenta* fungi (Figures 3b and c) after 4 weeks of incubation. But it was not the case for the exposition to *T. versicolor* after 12 weeks of incubation. There was a significant difference in the weight loss data between the treatments with or without humidity (Fig. 3a). These data are consistent with the results of Tjeerdsma et al. (2002), who found a good correlation between wood humidity and

strength of thermally modified radiate Pine and birch against some fungal species (*C. puteana*) while for some species, such as *T. versicolor*, there were no significant correlation between the wood humidity and the weight loss resistance. Thus humidity can affect positively the quality of the thermally modified wood, but it does not necessarily ensure a good resistance against fungal attack.

Conclusions

This study showed that increasing the thermal modification temperatures in the range of 190ºC to 220ºC increased the durability of *Populus tremuloides* against *T. versicolor*, *P. placenta* and *G. trabeum*. Significant reductions in weight loss of 84.2% to 100% were obtained for the fungi tested. In contrast, this wood was resistant to *C. puteana* fungal attack. Moreover, the holding time affected the weight loss of wood occurring due decay fungi. The heating rate had a limited effect on the weight loss, consequently, fungal attack of thermally modified wood. The humidity affected *T. versicolor* degradation. Weight losses of 14.2% and 32.7%, with and without humidity respectively, were detected after 12 weeks.

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FIGURE CAPTIONS

Fig.1. Effect of holding time on the weight loss of *Populus tremuloides* thermally modified at 210ºC after 4, 8 and 12 weeks exposure to: a) *T. versicolor* b) *G. trabeum* and c) *P. placenta.*

(**:**1h**:** boards heated to the maximum temperature and kept for 1 hour, : 3h: boards heated to the maximum temperature and kept for 3 hours)

Fig.2. Effect of heating rate on the weight loss of *Populus tremuloides* thermally modified at 210ºC after 4, 8 and 12 weeks exposure to: a) *T. versicolor* b) *G. trabeum* and c) *P. placenta.*

(: 15ºC/h: Heating rate of 15ºC per hour, :10ºC/h: Heating rate of 10ºC per hour)

Fig. 3 Effect of humidity on the weight loss of *Populus tremuloides* thermally modified at 210ºC after 4, 8 and 12 weeks exposure to: a) *T. versicolor* b) *G. trabeum* and c) *P.*

(: H: with humidity, : WH: without humidity)

Table 1 Summary of thermal modification parameters used in this study

Maximum treatment Temperature (ºC)	Heating Rate (ºC/h)	Holding Time (h)	Gas humidity (g water Vapor/m^3 dry gas)
190	15	1	100
200	15	1	100
210[a]	15[a]	1[a]	100[a]
210	15	3	100
210	10	1	100
210	15	1	0
220	15	1	100

[a] Base conditions

Table 2 Effect of thermal treatment temperature on decay resistance of Populus tremuloides to decay fungal after 12 weeks of colonisation

Fungal species	Heat treatment *Aspen*	MC (%)	SD* (±)	Weight loss (%)	SD* (±)	Reduction (%)
G. trabeum	Untreated	22.1	± 1.1	21.9	± 1.1	-
	190°C	5.6	± 1.5	19.9	± 1.5	9.1
	200°C	7.2	± 1.0	11.6	± 1.0	47.0
	210°C	19.9	± 1.1	12.4	± 1.1	43.4
	220°C	9.4	± 0.2	0.0	± 0.2	100
T. versicolor	Untreated	72.8	± 1.5	57.1	± 1.5	-
	190°C	5.8	± 1.5	26.3	± 1.5	53.9
	200°C	5.4	± 1.4	14.0	± 1.4	75.5
	210°C	7.6	± 1.3	21.3	± 1.3	62.7
	220°C	6.7	± 1.0	9.0	± 1.0	84.2
P. placenta	Untreated	10.2	± 1.6	36.7	± 1.6	-
	190°C	14.3	± 1.6	27.9	± 1.6	23.9
	200°C	21.0	± 1.6	19.7	± 1.6	46.3
	210°C	10.8	± 1.6	19.4	± 1.6	47.1
	220°C	0.0	± 0.0	0.0	± 0.0	100
C. puteana	Untreated	7.2	± 0.4	7.7	± 0.4	-
	190°C	15.3	± 0.5	7.1	± 0.5	7.8
	200°C	7.7	± 0.4	3.8	± 0.4	50.6
	210°C	15.4	± 0.8	3.5	± 0.8	54.5
	220°C	11.8	± 0.0	0.0	± 0.0	100

* SD: Standard Deviations

Fig. 1

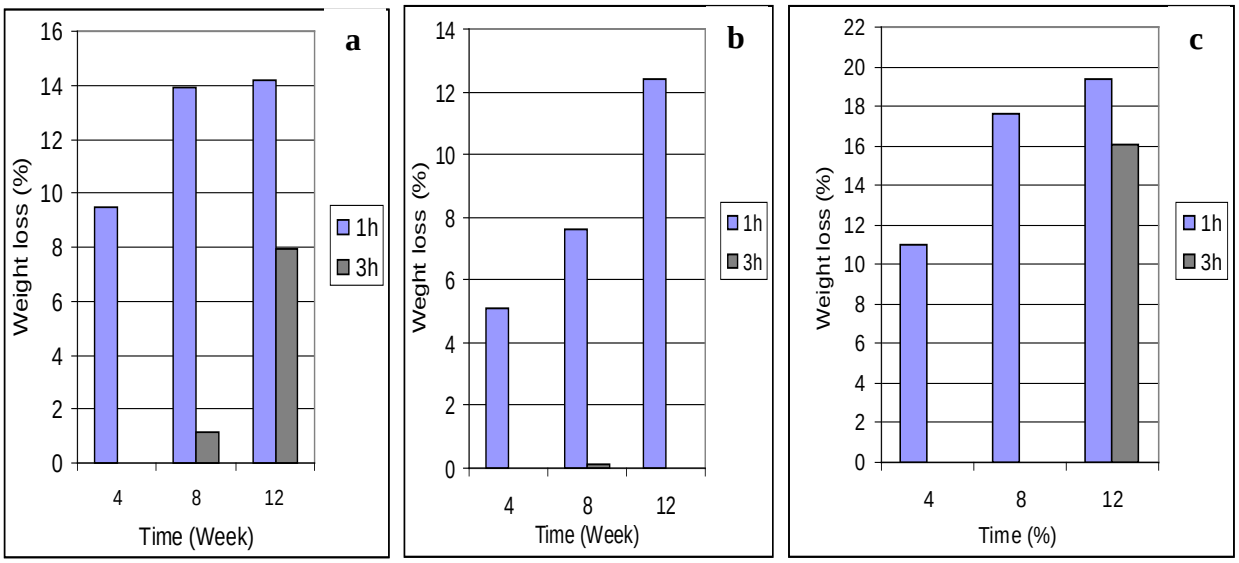

a
Weight loss (%)
16
14
12
10
8
6
4
2
0
4
8
12
Time (Week)
1h
3h
b
Weght loss (%)
14
12
10
8
6
4
2
0
4
8
12
Time (Week)
1h
3h
c
Weight loss (%)
22
20
18
16
14
12
10
8
6
4
2
0
4
8
12
Time (%)
1h
3h

Fig. 2

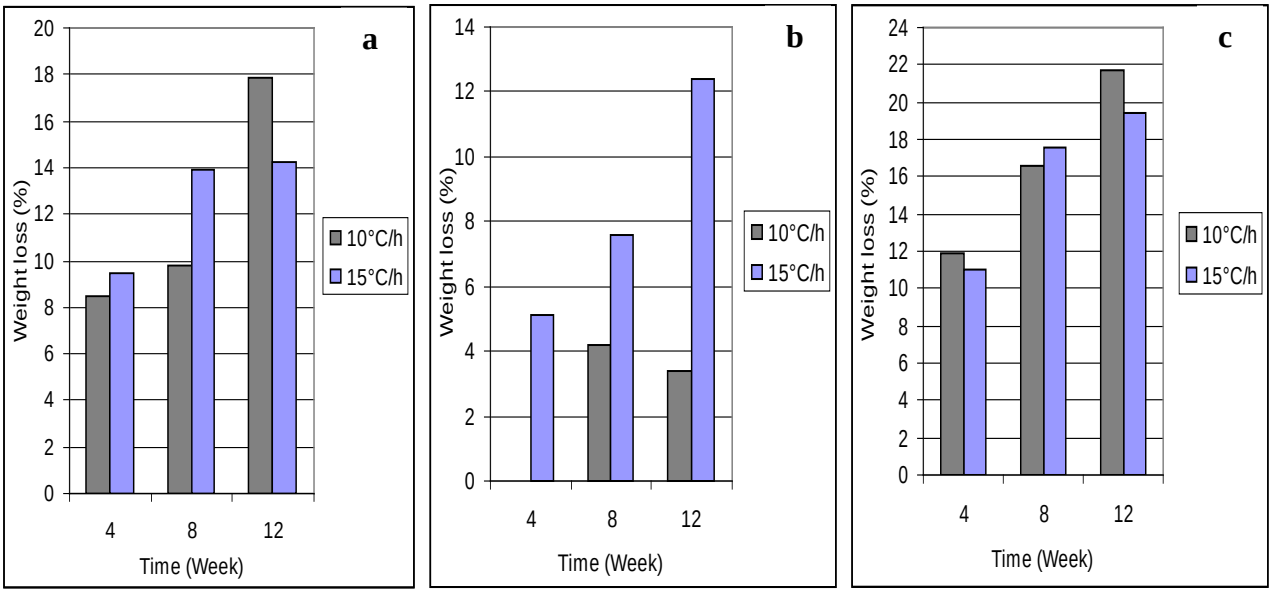
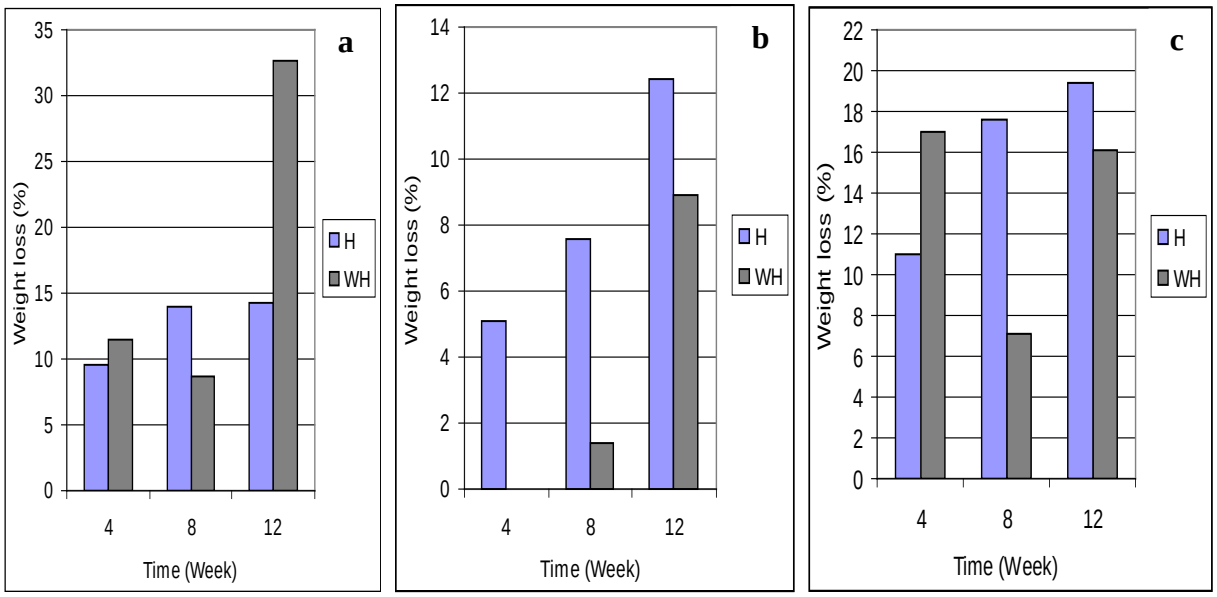

a
Weight loss (%)
Time (Week)
10°C/h
15°C/h
b
Weight loss (%)
Time (Week)
10°C/h
15°C/h
c
Weight loss (%)
Time (Week)
10°C/h
15°C/h

Fig. 3


a
Weight loss (%)
35
30
25
20
15
10
5
0
4
8
12
Time (Week)
H
WH
b
Weight loss (%)
14
12
10
8
6
4
2
0
4
8
12
Time (Week)
H
WH
c
Weight loss (%)
22
20
18
16
14
12
10
8
6
4
2
0
4
8
12
Time (Week)
H
WH