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SOME QUALITY CHARACTERISTICS INCLUDING ISOTHIOCYANATES CONTENT OF HORSERADISH CREAM AS AFFECTED BY STORAGE PERIOD

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Summary

During two-year experiments the effects of long-term storage at three temperature levels (2°C, 8°C and 18°C) on content of some nutritive components, including pungent compounds - isothiocyanates (ITC), in processed horseradish (cream) were studied. The significant changes of content of reducing sugars, total isothiocyanates and color indexes in horseradish cream after its storage were noted. The higher temperature of storage the higher content of reducing sugar in horseradish cream was observed. Content of allyl isothiocyanate (AITC) and especially phenylethyl isothiocyanate (PEITC) in fresh horseradish cream was lower in comparison to non-processed horseradish roots. Contents of both isothiocyanates decreased significantly during storage period. The highest decline of isothiocyanates level was observed during first four months of storage. The highest temperature of storage studied (18°C) caused faster decline of both isothiocyanates concentration in horseradish cream. To maintain the human health-promoting compounds in processed horseradish, as well as to keep sensory attributes (color), it is suggested to store horseradish cream in cold condition.

key words: *Armoracia rusticana* L., horseradish cream, storage, quality, allyl isothiocyanate, phenylethyl isothiocyanate

INTRODUCTION

Horseradish (*Armoracia rusticana* L.) is a perennial plant of the *Brassicaceae* family. It grows best in cool to moderate climates, flourishing in Northern, Middle and South-eastern Europe and in Northern America. The plant is probably native to southeastern Europe, but is popular around the world today, and is cultivated for its large white, tapered root.

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Horseradish roots contain high level of vitamin C and group of secondary metabolites - glucosinolates. Glucosinolates in plants are accumulated as a part of defense system against herbivores (Horbowicz *et al*. 2004). Their level and composition can be affected by many environmental factors (Ciska *et al*. 2000). Glucosinolates as biologically active substances found in horseradish and other plants of the family *Brassicaceae,* have generated considerable pharmacological interest due to their human health-promoting effects, particularly anti-carcinogenic properties (Talalay & Fahey 2001). Glucosinolates remain chemically stable within the cytoplasm until brought into contact with the enzyme myrosinase following tissue disruption (Mithen 2001). After physical damage of plant tissue, glucosinolates are broken down by myrosinase to variety of biologically active products (Bennett *et al*. 1996). Among their degradation compounds, the most abundant products in horseradish cream are isothiocyanates: allil (from sinigrin) and 2-phenylethyl isothiocyanates (from gluconasturtiin). Both isothiocyanates are largely responsible for the typical, characteristic flavours and pungency of mustard and horseradish (Mithen *et al*. 2000). Sinigrin (prop-2-enyl glucosinolate) and gluconasturtiin (2-phenylethyl glucosinolate) predominant glucosinolates found in horseradish roots are converted by myrosinase into allil- (AITC) and 2-phenylethyl isothiocyanates (PEITC) respectively (Fahey *et al.* 2001). In previous studies Horbowicz & Rogowska (2006) have found that level of AITC in roots of two types of horseradish cultivated in Poland was 3 to 10 times higher than PEITC.

Brassica vegetables due to high level of glucosinolates have some anti-carcinogenic properties (Stoewsand 1995, Horbowicz 2003). Isothiocyanates - main products of glucosinolates breakdown, inhibit mitosis and stimulate apoptosis in human tumor cells. The substances may selectively inhibit the growth of tumor cells even after initiation by chemical carcinogens (Sones 1984). Phenylethyl isothiocyanate inhibits the action of lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (McDanell & McLean 1988). According to Zhang (2001) isothiocyanates protect against tumor development in liver, mammary gland and forestomach. It has been suggested, that sinigrin may prevent cancer of the colon if foods containing it are eaten regularly (Munday 2002). Main pungent compound of horseradish - allyl isothiocyanate have been found to kill some bacterial strains (Lin *et al*. 2000). PEITC has been shown to inhibit induction of lung and esophageal cancer in both rat and mouse tumor models (Morse *et al.* 1993, Hecht 1996, Stoner & Morse 1997).

Horseradish roots after harvest are usually stored in cold room, before processing, up to next harvest. Commercial horseradish cream is kept on shelves in shops at different temperatures, although mainly at room temperature. Due to lack of information in available literature concerning the shelf life and changes of its quality, the aim of work presented was to study the effect of long-term storage on color indices and content of some horseradish constituents, especially isothiocyanates that affect pungency of horseradish cream commercially produced in Poland.

MATERIALS AND METHODS

The horseradish cream (commercial name "Łowicki") was produced from horseradish roots in 2005 and 2006 by "Bracia Urbanek" company, Łowicz, Poland. Production process consist of cleaning the horseradish roots with sodium metabisulfite, and then their mechanical disruption in vinegar (acetic acid) solution. Next mashed horseradish roots were mixed with egg powder, powdered milk, vegetable oil, sugar, salt, citric acid and were further packed in glass, closed jars (twist type). Horseradish cream used in studies was produced 2 weeks before starting of storage experiments.

Horseradish cream was stored for 8 months in tightly closed glass twist jars (200 g) in storage rooms at three temperature levels (2°C, 8°C and 18°C).

Every two months randomly chosen 5-jar samples of horseradish cream were taken for analysis of isothiocyanates, and after 8-months storage ascorbic acid, reducing sugars, total sugars and color measurements were carried out.

Contents of ascorbic acid (vitamin C) by titrimetric Tillmans method, total and reducing sugars by common Luff-Schoorl method were determined. Color indices (*L*, a*, b**) including the yellowness index (*YI*) according to Hunter *Lab* method were analyzed by MiniScan XE Plus colorimeter.

Isothiocyanates were analyzed according to method described by Sultana *et al.* (2002) with some modifications (Horbowicz & Rogowska 2006; Kosson & Horbowicz 2008). Briefly, 5 g samples of processed horseradish cream were homogenized with 200 mL of distilled water. The homogenates were left at room temperature for 2 hours to finish conversion of remaining glucosinolates to isothiocyanates by endogenous myrosinase. Obtained isothiocyanates were extracted from filtered slurry (5 cm^3) by extraction with methylene chloride (2 cm^3) containing 0.25 mg of internal standard (phenyl isothiocyanate). To separate organic and water layers mixtures were centrifuged for 5 minutes at 3000 g. Lower organic layer was withdrew from centrifuge vial using Pasteur pipette. Isothiocyanates were analyzed on gas chromatograph (Shimadzu GC 17A) equipped with flame ionization detector (FID); chromatographic conditions: column – 30 m x 0.32 mm I.D., stationary phase: HP-5 (5% crosslinked methylsilicone layer) 0.25 µm layer; column oven temperatures - initial: 40°C for 1 min., then increased to 100°C at rate 5°C$\cdot$min^{-1}; then increased to 240°C at rate 10°C$\cdot$min^{-1}; and held at 240°C for 10 minutes; carrier gas (helium) velocity: 56 cm$\cdot$s^{-1}; total pressure: 190 kPa; injection split ratio: 4:1; detector temperature: 250°C; and injector port temperature: 220°C. Results of isothiocyanates analysis were calculated on the basis of earlier prepared standard curves of allyl- and phenylethyl isothiocyanates.

Standards of allyl isothiocyanate (AITC) were purchased from BDH (UK), phenylethyl isothiocyanate (PEITC) from Aldrich-Sigma, and phenyl isothiocyanate (internal standard) from Merck - Schuchardt (Germany). Other solvents and reagents of appropriate purity were obtained from POCH, Gliwice (Poland).

Analyses were carried out in three replicates. Obtained results were analyzed by standard statistical procedure and the least significant differences calculated by Newman-Keuls test at P=0.05.

RESULTS AND DISCUSSION

The results of chemical composition analysis, including total isothiocyanates, ascorbic acid, reducing sugars and total sugars content, as well as color indexes of fresh horseradish cream and after its storage are given in Table 1 & 2. Horseradish cream (HC) produced in 2005 contained significantly more ascorbic acid (AA), than that from 2006 (Table 1 & 2). Level of ascorbic acid content in freshly produced horseradish cream was more than two times lower compared with horseradish roots (Kosson & Horbowicz 2008). Although the level of ascorbic acid in stored horseradish cream was low, the content 30 $mg \cdot 100g^{-1}$ fresh weight seems to be quite important for human diet in winter-spring season. Long-term storage caused significant decrease of AA in horseradish cream from year 2005, however did not affected its content during 2006 season. In both years the content of reducing sugars in freshly processed horseradish was significantly lower compared to stored one, and the higher temperature of storage enhanced accumulation of reducing sugars (Table 1 & 2). Probably increased level of the carbohydrates is result of di- and/or oligosaccharides hydrolysis during period of storage. The hydrolysis can be enhanced by presence of acetic and citric acids added during production of horseradish cream. On the other hand the similar tendency was observed during storage of horseradish roots (Kosson & Horbowicz 2008). It can be expected that hydrolytic enzymes are not deactivated during processing.

Table 1. Effect of long-term storage on the content of some components and color indices of horseradish cream; season 2005/2006

Analyzed component or parameter	Before storage	After 8 months storage at:		
		2°C	8°C	18°C
Ascorbic acid ($mg \cdot 100g^{-1}$ f.w.)	50.1 a	36.5 b	35.5 b	34.6 b
Reducing sugars ($g \cdot 100g^{-1}$ f.w.)	1.30 d	2.06 c	3.30 b	5.24 a
Total sugars ($g \cdot 100g^{-1}$ f.w.)	9.37 b	13.65 a	13.61 a	13.35 a
Whiteness (L*)	87.4 a	86.4 a	85.9 a	83.4 b
Redness (a*)	0.64 c	0.69 c	1.03 b	2.21 a
Yellowness (b*)	17.64 c	21.18 b	21.58 b	22.52 a
Yellowness index (YI)	35.94 c	38.80 b	39.89 b	43.30 a
Total isothiocyanates ($mg \cdot kg^{-1}$ f.w.)	631a	388 b	243 c	79 d

Note: Means in horizontal rows marked with various letters are significantly different according to Newman-Keuls test at P=0.05

Table 2. Effect of long-term storage on the content of some components and color indices of horseradish cream; season 2006/2007

Analyzed component or parameter	Before storage	After 8 months storage at:		
		2°C	8°C	18°C
Ascorbic acid (mg·100g^{-1} f.w.)	34.90 a	33.52 a	34.30 a	32.91 a
Reducing sugars (g·100g^{-1} f.w.)	1.00 d	2.28 c	4.06 b	7.39 a
Total sugars (g·100g^{-1} f.w.)	14.54 b	14.22 b	14.73 ab	15.44 a
Whiteness (L*)	85.4 a	85.3 a	85.2 a	83.4 b
Redness (a*)	0.58 d	0.82 c	1.17 b	2.33 a
Yellowness (b*)	20.1 b	22.0 a	22.2 a	22.5 a
Yellowness index (YI)	37.4 c	40.6 b	41.1 ab	43.4 a
Total isothiocyanates (mg·kg^{-1} f.w.)	645 a	303 b	168 c	22 d

Note: See Table 1.

There was no significant effect of storage temperature on total sugars levels in horseradish cream in 2005/2006 season. Index of whiteness (*L**) of freshly processed horseradish measured by Hunter *Lab* method was similar to horseradish stored at 2°C and 8°C, but yellowness (*b**) and yellowness index (*YI*) of horseradish was increased. The total content of isothiocyanates in stored horseradish was significantly lower than in freshly processed horseradish (Table 1 & 2).

The level of isothiocyanates is responsible for pungency of horseradish cream. Obtained results show that pungency of product stored at higher temperature (8°C and 18°C) was much lower compared to that stored at temperature 2°C or to fresh processed horseradish. However, on pungency of horseradish products have influence not only level of glucosinolates and isothiocyanates but myrosinase activity as well.

Similarly to row horseradish roots the only measurable isothiocyanates found in studied horseradish cream were allyl isothiocyanate (AITC) and phenylethyl isothiocyanate (PEITC). Wasabi (*Wasabia japonica* (Miq.) Matsum), plant similar to horseradish - is cultivated in different climate condition, and it does not contain phenylethyl isothiocyanate (PEITC) (Sultana *et al*. 2002, 2003). In earlier studies in which GC/MS method was applied, besides main isothiocyanates, in horseradish roots tissue a small amounts of 3-butenyl-ITC, 4-pentenyl-ITC, hexenyl-ITC, isopropyl ITC, sec-butyl ITC, n-propyl ITC, isobutyl ITC, n-butyl ITC and benzyl ITC were found (Uematsu *et al*. 2002). However, according to their studies, total level of the rare isothiocyanates did no exceeded 2.5% of contents of major isothiocyanates: AITC and PEITC.

Changes of content of allyl isothiocyanate (AITC) and phenylethyl isothiocyanate (PEITC) in horseradish cream during long term storage in 2005/2006 and 2006/2007 are presented on Fig. 1-4. Content of AITC in freshly processed horseradish reached level about 550 mg·kg^{-1} fresh weight (f.w.) in 2005 season and about 650 mg·kg^{-1} f.w. in 2006. Taking into account results of earlier studies (Kosson & Horbowicz 2008, Horbowicz & Rogowska 2006) the content of AITC in horseradish cream is only slightly lower in comparison to non-processed horseradish roots (700-750 mg·kg^{-1} f.w.), from which it was made. Such results could be expected, due to fact that endogenous myrosinase is the enzyme which converts appropriate glucosinolates into isothiocyanates. In fact level of the AITC and PEITC in horseradish cream depends on myrosinase activity in horseradish roots from which it was made (Xian & Kushad 2004, 2005).

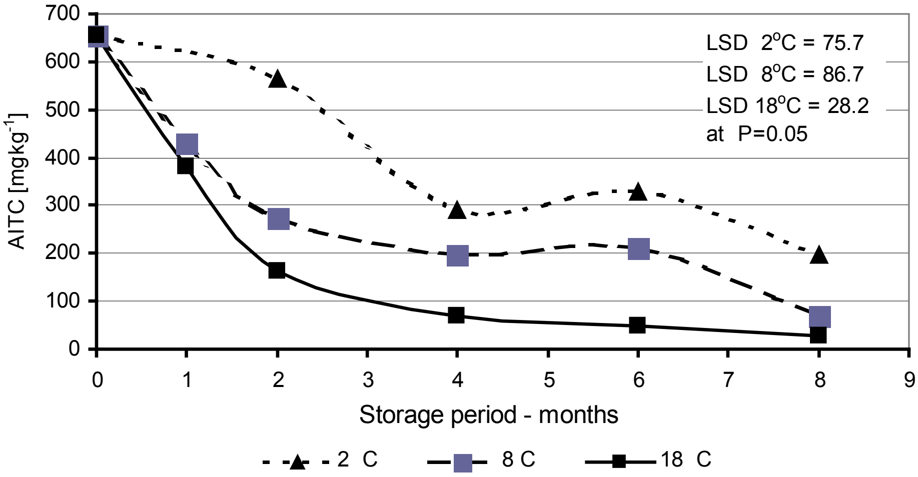

Fig. 1. Effect of storage temperature on allyl isothiocyanate (AITC) content [mg·kg^{-1} fresh weight] in processed horseradish; season 2005-2006

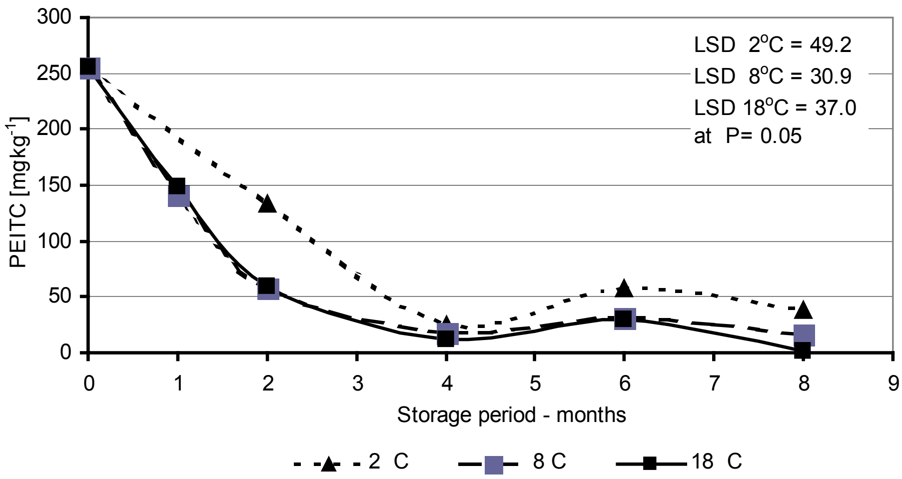

Fig. 2. Effect of storage temperature on phenylethyl isothiocyanate (PEITC) content [mg·kg^{-1} fresh weight] in processed horseradish; season 2005-2006

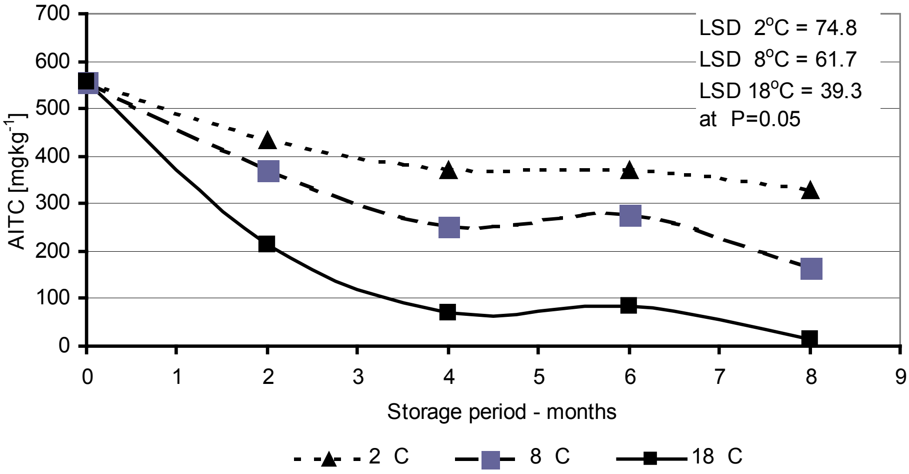

Fig. 3. Effect of storage temperature on allyl isothiocyanate (AITC) content [mg·kg^{-1} fresh weight] in processed horseradish; season 2006-2007

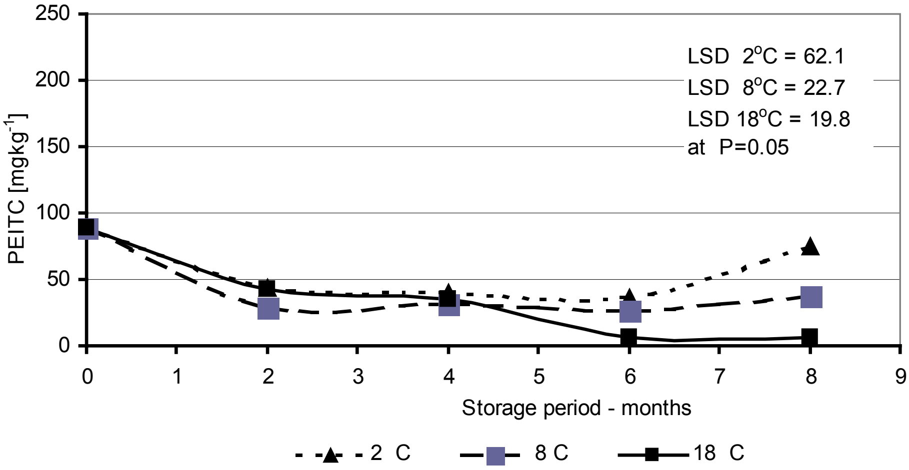

Fig. 4. Effect of storage temperature on phenylethyl isothiocyanate (PEITC) content [mg·kg^{-1} fresh weight] in processed horseradish; season 2006-2007

Concentration of PEITC in fresh horseradish cream was 2 to 5 times lower than AITC (Fig. 1-4). Moreover, concentration of PEITC in fresh horseradish cream in 2005 was two times higher than in season 2006. According to Ciska *et al.* (2000) variation in glucosinolates contents in horseradish between vegetation seasons can be attributed to weather conditions, mainly temperature during cultivation. It means that level PEITC in horseradish cream depends on gluconasturtiin content in horseradish roots. The gluconasturtiin is converted by myrosinase to PEITC during production of horseradish cream.

In both years of experiment, contents of both isothiocyanates decreased significantly during storage period (Fig. 1-4). The highest decline of isothiocyanates concentration was observed during first 4 months of storage. Also, the storage temperature significantly affected the isothiocyanates level in horseradish cream. The higher temperature of storage the more rapid decrease of isothiocyanates concentration was noted. It was especially apparent in case of allyl isothiocyanate (AITC) level, which is the main pungent compound in horseradish cream.

CONCLUSIONS

1. The level of storage temperature significantly affected the isothiocyanates content in horseradish cream. At lower temperature, 2°C and 8°C, isothiocyanates decomposition was significantly lower compared to temperature 18°C.
2. Due to intensity of biochemical processes occurring during long-term storage at temperature 18°C, horseradish cream has become significantly more yellow and dark compared to temperature storage 2°C or 8°C.
3. To maintain the human health-promoting substances content in processed horseradish, as well as to keep sensory attributes (color), it is suggested to store horseradish cream in cold condition.

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WPŁYW DŁUGOTRWAŁEGO PRZECHOWYWANIA NA JAKOŚĆ CHRZANU TARTEGO Z UWZGLĘDNIENIEM ZAWARTOŚCI IZOTIOCYJANIANÓW

Streszczenie

Przeprowadzono dwuletnie badania nad wpływem długotrwałego przechowywania w różnych temperaturach (2°C, 8°C i 18°C), na zawartość izotiocyjanianów i innych składników odżywczych w chrzanie tartym „Łowicki", pochodzącym z produkcji prze-

mysłowej firmy Bracia Urbanek w Łowiczu. Wystąpiły istotne zmiany zawartości cukrów redukujących, związków decydujących o ostrości chrzanu tartego (izotiocyjanianów). Wraz ze wzrostem temperatury przechowania chrzanu tartego zwiększał się poziom cukrów redukujących. Zawartości izotiocyjanianu allilu (AITC) i izotiocyjanianu fenyloetylu (PEITC) w chrzanie tartym były nieco niższe niż w surowcu, z którego został wyprodukowany. Podczas długotrwałego przechowania notowano istotny spadek zawartości AITC i PEITC w chrzanie tartym. Znacznie wyższe tempo spadku ich zawartości zaobserwowano podczas przechowania w temperaturze +18°C, w porównaniu do poziomu +2°C i +8°C. Ponieważ izotiocyjaniany allilu i fenyloetylu decydują o „ostrości" chrzanu tartego, dla zapewnienia jego odpowiedniej jakości produkt ten powinien być przechowywany w warunkach chłodniczych.