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STORAGE STABILITY OF PHYSICAL AND BIOCHEMICAL PARAMETERS OF PRESSURIZED AND HEAT TREATED *NAM PRIG NHUM* (THAI-GREEN-CHILI PASTE)

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Alteration of physical and chemical qualities of pressurized and heat treated *Nam Prig Nhum* (Thai-green-chili paste) was thoroughly assessed upon storage for eight weeks. During storage, colour L, –a*, and b* parameters of pressurized *Nam Prig Nhum* displayed comparatively low changes. The enzyme activities of peroxidase, lipoxygenase, and polyphenol-oxidase nearly fell by half during storage. Peroxidase was the most resistant to pressure followed by lipoxygenase and polyphenol-oxidase. For pungent components, all capsaicinoids in *Nam Prig Nhum* underwent alteration by the thermal processes as well as by acidic and oxidative degradation during storage. However, higher amount of capsaicinoids were retained in pressurized than in heat treated products.

Keywords: storage stability, Thai-green-chili paste, *Nam Prig Nhum*, pressurization, pasteurization, sterilization

Nam Prig Nhum (Thai-green-chili paste) is one of the favourite northern Thai sauces. It is a thick paste, moist, and fibrous comprising of baked green-chili, baked shallot, blanched garlic, and fish sauce. Conventional processes for preserving this cuisine commonly apply either pasteurization or sterilization. Although these processes provide the desired safety and shelf-life extension, they can dramatically impair basic sensorial attributes including colour, flavour, and texture of the products (Apichartsrangkoon et al., 2013). Therefore, a non-thermal technique such as ultra-high pressure could overcome this problem and provides an efficient preservation to reach the consumer requirement.

To develop the healthy pressurized products like green-chili paste, it is essential to study the magnitude of pressure denaturation of some enzymes, i.e. polyphenol-oxidase (PPO), peroxidase (POD), and lipoxygenase (LOX). In general, PPO catalyzes the oxidative reaction associated with undesirable browning, while POD is associated with off-flavour also off-colour of the products (Fujita et al., 1995). LOX causes chlorophyll destruction and off-flavour development (Eisenmenger & Reyes-de-Corcuera, 2009).

In general, pungent components (capsaicinoids) in chili are the major contributor of savoury of Thai cuisines. They consist of acid amides of vanillylamine and a C_8–C_{13} branched-chain fatty acid (Kobata et al., 1999) and could be degraded upon processing and storage, resulting loss of pungent flavour (Apichartsrangkoon et al., 2013). This study aimed to compare physicochemical qualities, particularly colour, pungent compounds, and enzyme

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activities, of pressurized (600 MPa/30 °C/20 min), pasteurized (90 ºC/3 min), and sterilized (121 °C/4 min) *Nam Prig Nhum* upon storage for eight weeks.

1. Materials and methods

1.1. Preparation of Nam Prig Nhum

Green-chili (*Capsicum annuum* Linn. var. *Jak Ka Pat*) and shallot (*Allium cepa* var. *Ascalonicum*) were baked at 210 °C for 20 min and 10 min, respectively, while garlic (*Allium sativum* L.) was blanched at 100 °C for 50 s. All ingredients, 65% green chili, 10% shallot and 15% garlic, were peeled, minced, and blended with 10% fish sauce for 5–10 min (Apichartsrangkoon et al., 2013).

1.2. Preparation of processed Nam Prig Nhum

All processed *Nam Prig Nhum* were prepared as described by Apichartsrangkoon and co-workers (2013). For pasteurization and sterilization, the packed *Nam Prig Nhum* was immersed in boiling water at 90±5 ºC for 3 min and was heated in a spray retort at 121 °C for 4 min (F_0=4), respectively. For pressurization, the packed samples were subjected to pressure 600 MPa at 30 °C for 20 min (Stansted Fluid Power, model 900, UK). The untreated, pressurized, and pasteurized *Nam Prig Nhum* were stored at 4 °C, while the sterilized samples were stored at 30 °C until use.

1.3. Microbiological assessments

The assessments of standard plate count, yeasts, and mounds of untreated and processed *Nam Prig Nhum* followed a modified method of the U.S. Food and Drug Administration, US FDA (2001).

1.4. Colour measurement

A colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan) was used to measure the colour of untreated and processed samples. Analytical data were expressed as Hunter L (lightness), a* (greenness/redness), and b* (yellowish/blueness) parameters.

1.5. Determination of peroxidase (POD), polyphenol-oxidase (PPO) and lipoxygenase (LOX) activities

Determination of activities of POD and PPO followed the spectroscopic method of Phunchaisri and Apichartsrangkoon (2005), measuring at λ_{max} 470 and 420 nm for 5 min, respectively, while determination of LOX activity followed the spectroscopic method of Gökmen and co-workers (2005), measuring at λ_{max} 234 nm for 5 min. One unit of enzyme activities was defined as an increase of 0.1 unit of absorbance per min (unit min^{-1}).

1.6. Determination of capsaicinoid contents

Capsaicinoids were determined by a modified liquid chromatography-mass spectrometry (LC-MS) method described by Reilly and co-workers (2001) and Apichartsrangkoon and co-workers (2013). The mass spectrometer was set for detecting the M+H positive ions of

nordihydrocapsaicin (m/z=294), capsaicin (m/z=306), dihydrocapsaicin (m/z=308), homocapsaicin (m/z=320), and homodihydrocapsaicin (m/z=322).

1.7. Data analysis

All data were the means of triplicate determinations with individual duplication (n=6). Analysis of variance and linear regression for the correlation of various parameters against storage time were carried out by SPSS Version 15.0 (SPSS Inc., Chicago, USA). Determination of significant differences among treatment means was done by Duncan's multiple range tests ($P \leq 0.05$).

2. Results and discussion

2.1. Microbiological qualities

Table 1 illustrates that untreated *Nam Prig Nhum* displayed general bacterial counts around 179 CFU g^{-1} and yeasts-moulds less than 10 CFU g^{-1} (data not shown). This meant that daily prepared *Nam Prig Nhum* complied with the regulation of Thai-Community-Product Standard (TCPS No. 293/2004) notifying that an acceptable *Nam Prig Nhum* should meet a standard microbial count below 4 log CFU g^{-1} and yeasts-moulds below 1 log CFU g^{-1} (Thai Industrial Standard Institute, 2004). However, within two weeks the microbes had proliferated over 10^6 and 10^2 CFU g^{-1} for standard plate counts and yeasts-moulds, respectively, indicating that this *Nam Prig Nhum* should not be kept more than a week at 4 °C. Therefore, the data of freshly prepared *Nam Prig Nhum* were shown only at the initial state in this investigation, regardless of the storage quality, while pressurized or pasteurized samples were stored up to eight weeks at refrigerated temperature, because their microbiological quality complied with the Thai regulation. However, increasing microbial counts of these batches at the final stages (Table 1) caused significant lowering of the pH of the products.

2.2. Colour parameters

Table 1 shows that colour L parameters for lightness of freshly made and pressurized *Nam Prig Nhum* were equivalent but significantly higher ($P \leq 0.05$) than those of pasteurized and sterilized samples. These suggested that high pressure could preserve colour better than thermal processes. Chaikham and Apichartsrangkoon (2012) also supported that pressurized (500 MPa/25 °C/30 min) longan juice had higher L parameter than fresh and pasteurized juices. However, after storing for 6 weeks, L parameters of pressurized and pasteurized *Nam Prig Nhum* significantly diminished ($P \leq 0.05$), while those of sterilized samples declined beyond week four. These results suggested that non-enzymatic browning was the primary cause of decreasing L parameters in sterilized samples but for those in mild processed batch, the change could be delayed, which were affirmed by lower reducing rates of the mild processed batches (shown by the slopes of their trend-lines).

The greenness or –a* of pressurized *Nam Prig Nhum* significantly declined ($P \leq 0.05$) from the first week and shifted to red region (a*) from the second week. For pasteurized and sterilized samples, slight red colour developed during processing and significantly increased ($P \leq 0.05$) their intensities during storage, which appeared beyond the second and fourth weeks for pasteurized and sterilized samples, respectively (Table 1). These results suggested that upon heating, Mg-dechelation took place in the chlorophyll molecule of thermal treated samples, altering the visual green to yellowish-green colour. On the other hand, the chlorophyll

Table 1. Microbiological qualities and colour values of *Nam Prig Nhum* storage up to 8 weeks at 4 °C for untreated, pressurized, and pasteurized samples and at 30 °C for sterilized samples

Parameters	Storage period (weeks)	Untreated samples	Mean values of processed *Nam Prig Nhum* by		
			Pressurization	Pasteurization	Sterilization
Total plate counts (log CFU g^{-1})	day one	2.3 ± 0.2^{Ab}	$<1^{Bd}$	$<1^{Bd}$	$<1^{B}$
	2	6.5 ± 0.6^{Aa}	$<1^{Bd}$	$<1^{Bd}$	$<1^{B}$
	4	–	1.3 ± 0.4^{Bc}	1.8 ± 0.4^{Ac}	$<1^{C}$
	6	–	2.3 ± 0.2^{Ab}	2.7 ± 0.2^{Ab}	$<1^{B}$
	8	–	3.4 ± 0.2^{Aa}	3.6 ± 0.3^{Aa}	$<1^{B}$
Colour parameters					
L parameter	day one	42.4 ± 0.5^{A}	42.5 ± 0.3^{Aa}	37.2 ± 1.4^{Ba}	30.9 ± 1.7^{Ca}
	2	–	42.1 ± 1.9^{Aab}	37.9 ± 2.2^{Ba}	28.7 ± 1.2^{Ca}
	4	–	41.6 ± 1.3^{Aab}	36.8 ± 2.0^{Ba}	28.6 ± 0.9^{Ca}
	6	–	41.2 ± 1.0^{Aab}	34.8 ± 2.9^{Bab}	26.7 ± 0.9^{Cb}
	8	–	39.7 ± 1.8^{Ab}	33.7 ± 1.9^{Bb}	25.3 ± 1.2^{Cb}
Slopes of linear trend-lines of L parameters VS storage			–0.3 (R^2=0.91)	–0.5 (R^2=0.82)	–0.7 (R^2=0.96)
a* parameter	day one	-1.7 ± 0.4^{C}	-1.5 ± 0.3^{Cd}	2.6 ± 0.2^{Bc}	11.4 ± 1.3^{Ab}
	2	–	-0.7 ± 0.1^{Cc}	3.3 ± 0.3^{Bab}	12.4 ± 1.1^{Ab}
	4	–	1.8 ± 0.3^{Bab}	3.5 ± 0.5^{Bab}	15.0 ± 1.6^{Aab}
	6	–	2.5 ± 0.4^{Ba}	3.6 ± 0.4^{Bab}	15.0 ± 1.1^{Aab}
	8	–	2.5 ± 0.4^{Ca}	4.9 ± 0.2^{Ba}	17.1 ± 1.2^{Aa}
Slopes of linear trend-lines of a* parameters VS storage			0.6 (R^2=0.89)	0.2 (R^2=0.86)	0.7 (R^2=0.95)
b* parameter	day one	19.2 ± 0.9^{C}	20.5 ± 1.2^{BC}	21.2 ± 1.2^{ABb}	23.5 ± 1.1^{Ac}
	2	–	20.4 ± 1.9^{B}	22.9 ± 1.5^{ABab}	23.4 ± 1.1^{Ac}
	4	–	20.7 ± 0.5^{B}	23.7 ± 0.9^{Aa}	25.3 ± 1.7^{Abc}
	6	–	21.5 ± 1.0^{C}	23.9 ± 0.9^{Ba}	26.3 ± 0.9^{Ab}
	8	–	22.2 ± 0.7^{C}	24.3 ± 1.3^{Ba}	28.0 ± 0.8^{Aa}
Slopes of linear trend-lines of b* parameters VS storage			0.2 (R^2=0.88)	0.4 (R^2=0.86)	0.6 (R^2=0.94)

Means in the same column or row followed by the same lowercase or capital letters respectively are not significantly different (P>0.05). Values presented are means of six replications

of pressurized samples was degraded by oxidative enzymes, such as POD or LOX, leading to loss of greenness on storage (Eisenmenger & Reyes-de-Corcuera, 2009). In overall, the sterilized product showed the highest rate of increasing a* parameter during storage followed by pressurized and pasteurized samples, indicated by the slopes of their trend-lines. Nevertheless, among the three processing techniques, pressurization brought about the least red colour development in *Nam Prig Nhum*.

Colour b* parameters for yellowish characteristic of pressurized *Nam Prig Nhum* were relatively stable (P>0.05) throughout the storage period, while those of pasteurized and

sterilized products significantly increased ($P \leq 0.05$) intensities beyond week two. Similar to a* parameter, the b* parameters of pressurized samples were comparatively lower than those of heat treated products and displayed the lowest increasing rate indicated by the slope of their trend-lines.

Overall, the loss of greenness or the increase of yellowish colour in *Nam Prig Nhum* was associated with chlorophyll degradation, along with the increase of Maillard browning especially in heat treated products. Maillard degradation usually takes place between alpha-amino groups and reducing sugars or ascorbic acid decomposition or destruction of pigments (Wang & Ho, 2008; Landl et al., 2010).

2.3. Enzyme activities

All enzymes in heat treated *Nam Prig Nhum* were completely inactivated, therefore, only activities of enzymes remaining in pressurized samples were considered. Figure 1 shows that among POD, PPO, and LOX, POD was the predominant enzyme remaining in the product, which was principally released from blanched garlic. This suggested that POD was the most resistant to pressure, followed by LOX and PPO. However, POD showed the highest reducing rate upon storage for 8 weeks (indicated by their slopes of trend-lines) and its activity declined from 78 to 44%; while LOX and PPO declined from 55 to 30% and 20 to 10%, respectively. These affirmed that nearly half of all enzyme activities diminished upon storage, which might be attributable to enzyme forming temporary complex with available substrates such as capsaicinoids or phenols in green chili, enabling loss of pungency (Skrede et al., 2000). It was noteworthy that there were residual enzymes in *Nam Prig Nhum* liberated from baked shallot and blanched garlic, since enzyme in baked chili was completely inactivated upon baking at the initial preparation (Srisajjalertwaja et al., 2012).

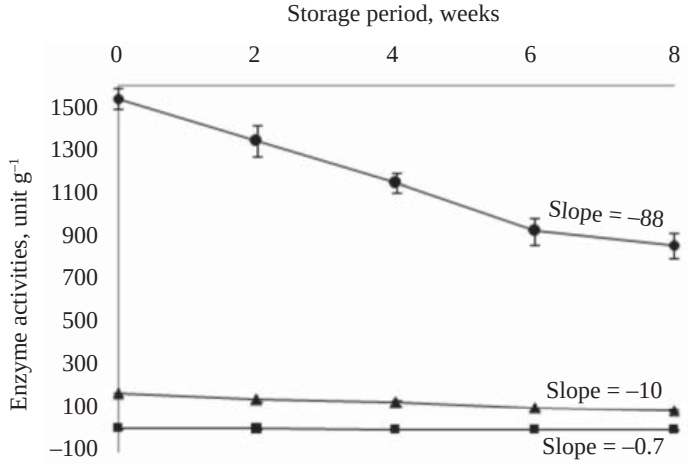

Fig. 1. Enzyme activities of pressurized *Nam Prig Nhum* stored at 4 °C for 8 weeks ●: POD; ▲: LOX, and ■: PPO

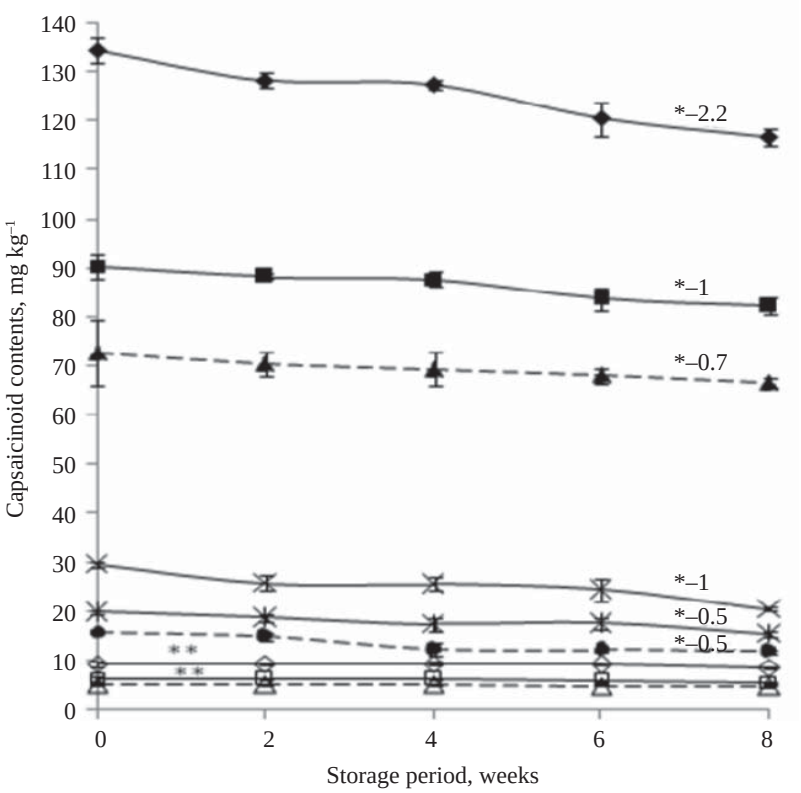

Fig. 2. Capsaicinoid contents in *Nam Prig Nhum* stored at 4 °C for 8 weeks and sterilized samples stored at 30 °C. * slope, ** slope = –0.1; ◆: Capsaicin in pressurized samples; ■: capsaicin in pasteurized samples; ▲: capsaicin in sterilized samples; ×: dihydrocapsaicin in pressurized samples; *: dihydrocapsaicin in pasteurized samples; ●: dihydrocapsaicin in sterilized samples, ◇: nordihydrocapsaicin in pressurized samples; □: nordihydrocapsaicin in pasteurized samples; △: nordihydrocapsaicin in sterilized samples

2.4. Capsaicinoid contents

Capsaicinoids comprise two main moieties. The vanillylamide moiety derives from phenylalanine, while the branched fatty acid moiety originates from *L*-valine or *L*-leucine (Schweiggert et al., 2006). Five major derivatives of capsaicinoids were found in this study of which capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were three primary capsaicinoids accounting for 95.7% of the total capsaicinoids. Upon baking at 210 °C for 20 min, around 76.3% capsaicinoids remained in the baked chili. The loss of capsaicinoids was due to an impact of heat dissociating the vanillylamide moiety from the branched fatty acid moiety at m/z 137 (Apichartsrangkoon et al., 2013). When the baked chili was incorporated in *Nam Prig Nhum*, these capsaicinoids would undergo further reduction by pasteurization or sterilization as well as by acidic and oxidative degradation during storage. Figure 2 shows that in comparison with thermal processes, pressurization provided the highest remaining amount of capsaicinoids, which decreased from the second week of storage, except for nordihydrocapsaicin that decreased at week 8. However, at the final week of storage, 66–88, 50–59, and 39–48% of capsaicinoids remained in the pressurized, pasteurized, and sterilized

Nam Prig Nhum, respectively. Additionally, capsaicin and nordihydrocapsaicin in heat treated samples diminished from week 6, whereas dihydrocapsaicin decreased from week 2 and onward. The loss of capsaicinoids during storage might be caused by oxidative enzymes, especially POD, and the acidic environment created by bacteria or by the bacteria themselves (Shrivastava & Saxena, 2011). Although, capsaicinoids of pressurized *Nam Prig Nhum* showed higher reducing rate during storage than those of heat treated samples indicated by the slopes of their trend-lines, the highest amount of these pungent components still remained in the pressurized products throughout the storage period, suggesting that pressurization could better preserve all capsaicinoids than in the thermal processes.

3. Conclusions

The standard plate counts indicated that general microbes in *Nam Prig Nhum* were satisfactorily eliminated in correspondence with the decrease of product pH. Upon storage, colour L, a*, and b* parameters of pressurized *Nam Prig Nhum* displayed the lowest rate of alteration, while those of sterilized batch showed the highest rate of degradation. For enzyme activities, POD was the most resistant to pressure, followed by LOX and PPO, and nearly half of these enzymes diminished by the final week. For pungent components, all capsaicinoids were reduced by acidic and oxidative degradation during storage. However, higher amount of capsaicinoids were retained in pressurized than in heat treated products.

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