Changes of Hormone Sensitive Lipase, Adipose Tissue Triglyceride Lipase, and Free Fatty Acids in Subcutaneous Adipose Tissues throughout the Ripening Process of Dry-cured Ham

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Summary and Implications

Changes in adipose triglyceride lipase (ATGL) and hormone sensitive lipases (HSL) in subcutaneous adipose tissue of Xuanwei ham at different ripening stages were studied. Green hams were salted for 40 d and then ripened in a ventilated chamber for 15 months. Adipose triglyceride lipase and HSL could be detected during the whole ripening period of Xuanwei ham, but their protein levels decreased as the ripening time increased. The decrease of lipase proteins could be attributed to the decrease in protein synthesis as ripening time progressed. The level of free fatty acids (FFAs) increased at the first stage but HSL protein remained constant indicating that HSL was more important than ATGL for adipose tissues lipolysis. The main FFAs produced during the ripening period of Xuanwei ham were oleic acid, linoleic acid and palmitic acid, suggesting that the triacylglycerols containing these three FFAs were preferential substrates for ATGL and HSL.

Introduction

Adipose tissues in dry-cured hams play important roles in flavor development and significantly contribute to the nutritional value of ham. In addition, the color, firmness and thickness of adipose tissues can affect consumer's acceptance of dry-cured ham. During the long process under mild ripening conditions of dry-cured ham, lipids undergo intense lipolysis that involves a set of endogenous enzymes followed by auto-oxidation, which contributes to the formation of aromatic volatile compounds. Thus, endogenous lipolytic enzymes are very important for flavor development and final flavor quality of ham. Two main lipase systems are involved in the lipolysis of subcutaneous adipose tissues: neutral and basic lipases described as hormone sensitive lipases (HSL) and lipoprotein lipases (LPL), respectively. By controlling these enzymes, therefore, we may be able to standardize processing procedure and improve the quality of dry-cured ham. However, only few studies have been conducted on the enzymes responsible for the lipolysis in dry-cured ham. In recent years, a novel lipase named ATGL (adipose triglyceride lipase, also called desnutrin), which plays a key role in triacylglycerol (TAG) breakdown (lipolysis) in adipocytes, was discovered. This new and exciting finding challenges the concept that is widely accepted for more than 30 years: HSL is the rate-limiting enzyme responsible for triacylglycerol breakdown. This also suggests that both ATGL and HSL are the major lipases involved in the lipolysis of dry-cured ham during processing. The objective of this study was to analyze adipose triglyceride lipase profiles during Xuanwei ham maturation in order to understand the lipolysis of dry-cured hams better.

Materials and Methods

Dry-cured hams were produced using meats from Wujin X Yorkshire cross-bred pigs. The green hams were held at 1-4 ºC and 50-60% relative humidity (RH) for 10-12 h. Then, hams were salted for three times, 2.5%, 3.5%, and 2% (w/w) respectively, and the whole duration of salting was about 40 d at 1-7 °C and 70-80% RH. After soaking in cold water for 6-8 h and washing the excess salt on the surface of the hams, they were hung on strings in a ventilated chamber and ripened for 15 months. The external subcutaneous adipose tissues covering the *Biceps femoris* muscle were removed from six hams at 0, 3, 6, 9 and 15 months of ripening period, vacuum-packaged in oxygen impermeable bags (O_2 permeability, 9.3 mL $O_2/m^2/24$ h at 0 °C) and stored at -80 °C until analyzed. Adipose tissue HSL and ATGL protein level, and fatty acids were analyzed.

Results and Discussion

3.1 Adipose tissue HSL and ATGL protein levels as affected by processing factors

According to recent reports, HSL, largely found in cytosol, hydrolyzes diacylglycerol (DAG) to generate monoacylglycerol (MAG). In current study, HSL could be detected throughout the 15 months of ripening period (Fig. 1A). During the first 3 months of ripening period, HSL protein level decreased slowly, and then decreased rapidly (41.94%, $p < 0.01$) between 3 and 6 months. From 9 to 15 months, HSL level sharply decreased (88.64%, Fig. 1A). ATGL could be detected up to 15 months of ripening period (Fig. 1B). The rapid decrease (42%) in ATGL ($p < 0.01$) was observed during the first ripening phase (0 - 3 months ripening period), and significantly decreased (29.31%) during the second ripening phase (3 - 6 months). It decreased at a slower rate during the third ripening phase (6 - 9 months) while a rapid decrease (60.66%) was observed

at the final phase (9 - 15 months). Evidences from *in vitro* studies showed that ATGL together with HSL was quantitatively the most important lipases found in adipose tissues. They were responsible for more than 95% of the TAG hydrolysis activity while other lipases seemed to just play a minor role in fat cell lipolysis. It is, therefore, suggested that these two lipases are mainly responsible for the FFA accumulation during ham processing. Table 1 showed that the amount of FFAs increased significantly during the whole sampling period from 60.55 to 110.07 mg g^{-1} ($p < 0.05$), and the kinetics of FFAs release were consistent with ATGL and HSL protein levels during ripening. During the first stage of process (0 - 3 months) when ATGL and HSL had the maximum protein levels, the amount of FFAs increased 29.51% (from 60.55 to 78.42 mg g^{-1}, $p < 0.01$, Table 1 and Fig. 1). The rate of FFAs increase at the later stages (from 6 to 15 months of ripening period) was lower than that of the earlier stages, which were attributed partly to the lower lipase levels during the later stages. In this study, even at the last ripening phase (9 - 15 months), we found increased accumulation of FFAs, which were consistent with our findings that the two lipases were detected and most possibly had activities at that time.

Data in Table 2 indicate that during the first sampling stage (0 - 3 months ripening), the pH decreased significantly (from pH 6.06 to 5.76, $p < 0.01$), and then remained relatively constant during most of the aging period before sharp decrease (42.96%) at the last phase. It is well known that proteins precipitate if the pH is close to their isoelectric point. A recent study indicated that the isoelectric point of TG-lipase from adipose tissues is 5.8-6.0. This can, in some degree, explain the decrease of HSL and ATGL protein levels in later stages of ripening (Fig. 1). Also, the low pH at the later stages of ripening would inactivate the two lipases. Compared with the first sampling stage (0 - 3 months), a_w decreased dramatically (0.8, $p < 0.01$, Table. 2) and NaCl concentration increased significantly (0.69%, $p < 0.01$, Table. 2) after 6 months of ripening. This means that hams experienced a relatively strong dehydration and salt penetration after 6 months of dry-curing. Although, both the a_w and NaCl concentration slightly changed during the whole ripening period (a_w, from 0.89 to 0.80; NaCl concentration, from 0.55% to 0.69%). It is indubitable that enzymes are usually more soluble in dilute salt solutions than in pure water. When the salt concentration is high enough, the proteins will be sufficiently dehydrated to lose solubility. These may be another reason contributing to the decrease of HSL and ATGL protein levels as the ripening time progressed.

The results showed that ATGL and HSL could be detected during the 15 months of ripening period. Further research is needed to develop appropriate methods to measure the activity of HSL and ATGL, and find the correlation between lipase activity and flavor development in dry-cured hams.

3.2 Preferential substrates of HSL and ATGL

Oleic acid (C18:1, ranging from 28.37 to 48.98 mg g^{-1}), palmitic acid (C16:0, ranging from 10.67 to 26.66 mg g^{-1}), and linoleic acid (C18:2, ranging from 9.47 to 14.26 mg g^{-1}) were the major FFA produced during Xuanwei ham maturation (Table 1). This indicated that triacylglycerols containing these three fatty acids such as POL, OOL and POO might be easier targets for lipolysis, and most likely, they were preferred substrates for ATGL and HSL. Until now, little is known about the specificity of endogenous lipases of adipose tissues, and the main substrates for HSL and ATGL are not clearly known. However, some researchers showed that ham showed preferential losses of POL (palmitoyl-oleoyl-linoleoyl glycerol), POO (di-oleoyl-palmitoyl glycerol) and OOL (di-oleoyl- linoleoyl glycerol) during processing. Additionally, others reported that the rank order of substrate preference for adipose tissue HSL was: polyunsaturates > monoenes > saturates, demonstrating that *in vivo* HSL might prefer long-chain unsaturated fatty acids as the substrates. Thereby, it contributed to the observed preferential mobilization of some highly unsaturated fatty acids.

Figure. 1. Western blotting analysis of ATGL and HSL protein contents in adipose tissue during Xuanwei ham maturation, $p<0.01$, n = 6. (A) ATGL relative protein expression in adipose tissue during Xuanwei ham maturation; (B) HSL relative protein expression in adipose tissue during Xuanwei ham maturation.

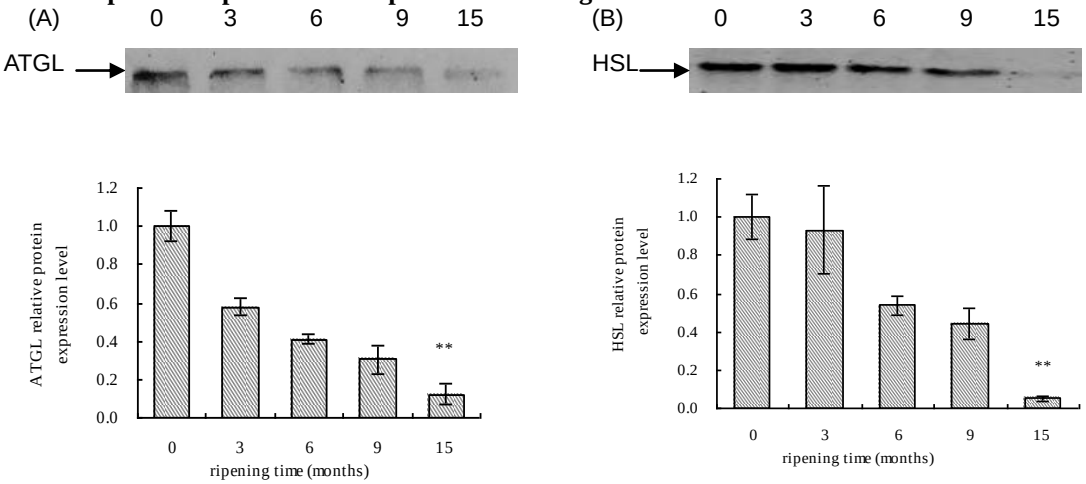

Table 1. Changes in pH, a_w and NaCl content in adipose tissue during Xuanwei ham ripening [a]

Ripening time (months)	0	3	6	9	15	Sig.[b]
pH	6.06±0.06a	5.76±0.16bc	5.71±0.12bc	5.82±0.11b	5.57±0.15c	**
a_w	0.89±0.021a	0.88±0.014ab	0.82±0.019ab	0.80±0.014b	0.80±0.020b	**
NaCl (%)	0.55±0.058	0.55±0.027	0.65±0.04	0.63±0.035	0.69±0.019	ns

[a]The values are mean ± standard deviation. n = 6.
[b]Sig. significance; on the same row, means with different letters differ significantly. Significance levels: ns, not significant, ** $p<0.01$,

Table 2. Free fatty acids changes in adipose tissue during Xuanwei ham maturation [a, b]

Ripening time (months)	0	3	6	9	15	Sig.[c]
Free fatty acids						
C12:0	0.78±0.50	0.79±0.49	0.69±0.15	0.54±0.17	0.66±0.07	ns
C14:0	1.07±0.39b	1.90±0.12ab	2.79±0.67a	2.19±0.51ab	2.41±0.69a	*
C16:0	10.67±3.20b	16.22±1.65ab	21.97±4.35ab	21.02±2.88ab	26.66±6.62a	**
C18:0	4.15±1.60b	4.95±0.75ab	5.49±0.94ab	5.16±0.65ab	7.18±0.81a	*
C20:0	0.85±0.39	1.41±0.21	1.10±0.18	1.27±0.50	1.53±0.16	ns
Total saturated	17.53±5.63b	25.27±1.72ab	32.03±6.22ab	30.18±3.70ab	38.45±8.26a	**
C16:1	2.04±0.55	2.57±0.14	4.08±0.74	3.64±1.13	3.75±0.94	ns
C18:1	28.37±7.23c	33.81±5.95bc	40.75±5.32abc	45.54±2.37ab	48.98±7.74a	*
C20:1	1.28±0.55	1.27±0.17	1.20±0.08	0.91±0.35	0.79±0.12	ns
Total monounsaturated	31.69±8.18b	37.65±6.12ab	46.04±5.95ab	50.09±3.10a	53.51±8.79a	*
C18:2	9.47±2.11	12.19±1.18	11.24±1.78	15.20±5.07	14.26±3.48	ns
C18:3	0.88±0.26b	2.24±0.53ab	2.02±1.03ab	3.56±0.45a	3.08±1.09ab	**
C20:4	0.97±0.60	1.07±0.68	0.81±0.22	0.78±0.26	0.76±0.24	ns
Total polyunsaturated	11.33±2.95	15.49±0.71	14.07±0.60	19.54±5.09	18.12±4.77	ns
Total free fatty acids	60.55±16.69b	78.42±7.80ab	92.14±11.80ab	99.80±11.60a	110.07±21.81a	*

[a]Results are expressed as mg g^{-1} lipid. n = 6. [b]The values are mean ± standard deviation.
[c]Sig. significance; on the same row, means with different letters differ significantly. Significance levels: ns, not significant; * $p<0.05$; ** $p<0.01$.